

***Rhodotorula rubra* CBS 6469**
Mediated Reduction of
Carbon-Carbon Double Bonds.

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Contents.

<i>Background and Aims.</i>	<i>vii</i>
<i>Abstract.</i>	<i>ix</i>
<i>Acknowledgements.</i>	<i>x</i>
<i>Abbreviations.</i>	<i>xi</i>
 1. INTRODUCTION.	 1
1.1 Introduction To Biotransformations.	1
1.2 Advantages and Disadvantages of Biocatalysis.	2
1.3 Using Biocatalysis : Whole Cell Sytems or Isolated Enzymes ?	4
1.4 Classes of Enzyme.	5
1.5 Reduction of Carbonyl Groups.	7
1.6 Reduction of Carbon-Carbon Double Bonds.	10
1.6.1 General Points.	10
1.6.2 Yeast Reductions.	10
1.6.2.1 Reduction of Cinnamaldehydes and Cinnamyl Alcohols.	10
1.6.2.2 Reduction of α,β -Unsaturated Ketones.	16
1.6.2.3 Reduction of α,β -Unsaturated Aldehydes and Allylic Alcohols.	22
1.6.2.4 Reduction of Halogenated Olefins.	24
1.6.2.5 Reduction of α,β -Unsaturated Esters.	26
1.6.2.6 Reduction of α,β -Unsaturated Lactones.	29
1.6.2.7 Reduction of Sulfur-Substituted Olefins.	32
1.6.2.8 Reduction of Nitro-olefins.	33
1.6.2.9 Miscellaneous.	37
1.6.3 Reduction By Other Systems.	38
1.6.3.1 <i>Beauveria bassiana</i> .	38
1.6.3.2 <i>Clostridium</i> spp..	39
1.6.3.3 <i>Nicotiana tabacum</i> .	41
1.6.3.4 <i>Streptomyces cinereocrocatus</i> .	42
1.6.3.5 <i>Corynebacterium equi</i> IFO 3730.	42
1.6.4 Metabolic Pathways.	43
 2. RESULTS & DISCUSSION : SYNTHESIS OF POTENTIAL SUBSTRATES.	 45
2.1 Synthesis of Potential Substrates Based Upon 5-Benzylidene Thiazolidine-2,4-dione.	46
2.1.1 Knoevenagel Condensation of Various Benzaldehydes and Benzophenones With Five Membered Heterocycles.	46
2.1.2 Protection of Ring Nitrogen.	50

2.1.3 Regio-chemistry of Benzyldiene Compounds.	51
2.2 Synthesis of Non-Commercially Available Aldehydes.	54
2.2.1 Synthesis of 4-Iodobenzaldehyde 209 and (Z)-5-(4-methoxybenzyldiene- <i>d</i>) thiazolidine-2,4-dione 215 .	54
2.2.2 Synthesis of 4-Propyl and 4-Butylbenzaldehyde, 216 and 217 .	56
2.2.3 Synthesis of 4- <i>i</i> -Propoxy and 4- <i>i</i> -Butoxy Benzaldehyde, 218 and 219 .	57
2.2.4 Synthesis of 4-Benzylbenzaldehyde 222 .	57
2.3 Synthesis of the Benzyldiene Precursor to Englitazone and its Isomer, <i>R</i>- and <i>S</i>-231.	58
2.4 Synthesis of (Z)-5-[(2-benzoyl-5-benzofuranyl)methylidene] thiazolidine-2,4-dione 235.	62
2.5 Expanding the Template.	63
2.5.1 Knoevenagel Condensation Between Cyclohexane Carboxaldehyde and Thiazolidine-2,4-dione.	64
2.5.2 Ring-Opening of 196 .	64
3. RESULTS & DISCUSSION : BIOTRANSFORMATION OF SUBSTRATES.	67
3.1 Development of Biotransformation Conditions.	67
3.2 Preparative Scale Biotransformations.	71
3.3 Screen of Potential Substrates Against <i>Rhodotorula rubra</i> CBS 6469.	74
3.4 Biotransformations With Dried and Immobilised <i>Rhodotorula rubra</i> CBS 6469.	85
3.4.1 Lyophilisation of <i>Rhodotorula rubra</i> CBS 6469.	86
3.4.2 Preparation of an Acetone Powder of <i>Rhodotorula rubra</i> CBS 6469.	86
3.4.3 Immobilisation by Gel Entrapment in Calcium Alginate.	87
4. RESULTS & DISCUSSION : CHEMICAL REDUCTION.	90
4.1 Reduction of Tri-substituted Double Bonds.	90
4.1.1 Hydrogenation.	90
4.1.2 Other Methods.	93
4.2 Reduction of Tetra-substituted Double Bonds.	94
5. RESULTS & DISCUSSION : BIOTRANSFORMATION MECHANISM.	97
5.1 Biotransformation of (Z)-5-(4-methoxybenzyldiene- <i>d</i>) thiazolidine-2,4-dione 215 .	97
5.2 Biotransformation of (Z)-5-(4-methoxybenzyldiene)thiazolidine-2,4-dione 172 in Deuterated Medium.	100
5.3 Attempted Biotransformations to Afford Enantiomerically Enriched Product.	109
5.4 Conclusions.	111

6. EXPERIMENTAL : SYNTHESIS.	115
6.1 General Experimental.	115
6.2 Knoevenagel Condensation Between Thiazolidine-2,4-dione and 4-Substituted Benzaldehydes.	118
6.2.1 General Procedures.	118
6.2.2 (Z)-5-benzylidenethiazolidine-2,4-dione 170 .	119
6.2.3 (Z)-5-(4-hydroxybenzylidene)thiazolidine-2,4-dione 171 .	120
6.2.4 (Z)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione 172 .	120
6.2.5 (Z)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione 173 .	121
6.2.6 (Z)-5-(4- <i>n</i> -propoxybenzylidene)thiazolidine-2,4-dione 174 .	121
6.2.7 (Z)-5-(4- <i>n</i> -butoxybenzylidene)thiazolidine-2,4-dione 175 .	122
6.2.8 (Z)-5-(4-benzyloxybenzylidene)thiazolidine-2,4-dione 178 .	123
6.2.9 (Z)-5-(4-methylsulfanylbenzylidene)thiazolidine-2,4-dione 179 .	123
6.2.10 (Z)-5-(4-methylbenzylidene)thiazolidine-2,4-dione 180 .	124
6.2.11 (Z)-5-(4-ethylbenzylidene)thiazolidine-2,4-dione 181 .	125
6.2.12 (Z)-5-(4-fluorobenzylidene)thiazolidine-2,4-dione 185 .	125
6.2.13 (Z)-5-(4-chlorobenzylidene)thiazolidine-2,4-dione 186 .	126
6.2.14 (Z)-5-(4-bromobenzylidene)thiazolidine-2,4-dione 187 .	127
6.2.15 (Z)-5-(4-cyanobenzylidene)thiazolidine-2,4-dione 189 .	127
6.2.16 (Z)-5-(4-nitrobenzylidene)thiazolidine-2,4-dione 190 .	128
6.2.17 (Z)-5-(4-trifluoromethoxybenzylidene)thiazolidine-2,4-dione 191 .	128
6.3 Knoevenagel Condensation Between 4-Substituted Benzaldehydes and Other Five-Membered Heterocycles.	129
6.3.1 (Z)-5-(4-methoxybenzylidene)imidazolidine-2,4-dione 192 .	129
6.3.2 (Z)-5-(4-chlorobenzylidene)imidazolidine-2,4-dione 193 .	130
6.3.3 (Z)-5-(4-cyanobenzylidene)imidazolidine-2,4-dione 194 .	131
6.3.4 (Z)-5-(4-methoxybenzylidene)thiazolidine-2-imine-4-one 195 .	132
6.3.5 (Z)-5-(4-methoxybenzylidene)thiazolidine-2-thione-4-one 196 .	133
6.4 Knoevenagel Condensation Between Thiazolidine-2,4-dione and 2-, 3-, Di- or Tri-substituted Benzaldehydes.	134
6.4.1 General Procedure.	134
6.4.2 (Z)-5-(2-methoxybenzylidene)thiazolidine-2,4-dione 197 .	135
6.4.3 (Z)-5-(3-methoxybenzylidene)thiazolidine-2,4-dione 198 .	135
6.4.4 (Z)-5-(2,6-dimethoxybenzylidene)thiazolidine-2,4-dione 199 .	136
6.4.5 (Z)-5-(3,5-di- <i>t</i> -butyl-4-hydroxybenzylidene)thiazolidine-2,4-dione 200 .	136
6.5 Knoevenagel Condensation Between Thiazolidine-2,4-dione and 4-Substituted Benzophenones.	137
6.5.1 General Procedure.	137
6.5.2 (Z)-5-(1-methyl-1-phenylmethylidene)thiazolidine-2,4-dione 201 .	138
6.5.3 (Z)-5-(1-methyl-1-(4-methoxyphenyl)methylidene)thiazolidine-2,4-dione 202 .	138
6.5.4 (Z)-5-(1-ethyl-1-(4-methoxyphenyl)methylidene)thiazolidine-2,4-dione 203 .	139
6.6 Synthesis Of (Z)-5-(4-substitutedbenzylidene)-3-substituted thiazolidine-2,4-diones.	140
6.6.1 General Procedure.	140
6.6.2 (Z)-3-methyl-5-(4-methoxybenzylidene)thiazolidine-2,4-dione 204 .	140
6.6.3 (Z)-3-benzyl-5-(4-methoxybenzylidene)thiazolidine-2,4-dione 205 .	141
6.7 Synthesis of (Z)-5-(4-iodobenzylidene)thiazolidine-2,4-dione.	142
6.7.1 2-Iodoxy benzoic acid 210 .	142
6.7.2 4-Iodobenzoic acid methyl ester 207 .	142

6.7.3 4-Iodobenzyl alcohol 208.	143
6.7.4 4-Iodobenzaldehyde 209.	144
6.7.5 (Z)-5-(4-iodobenzylidene)thiazolidine-2,4-dione 188.	145
6.8 Synthesis Of (Z)-5-(4-methoxybenzylidene-d)thiazolidine-2,4-dione.	146
6.8.1 4-Methoxy benzoic acid methyl ester 212.	146
6.8.2 4-Methoxy benzyl alcohol- <i>d</i> ₂ 213.	147
6.8.3 4-Methoxy benzaldehyde- <i>d</i> 214.	148
6.8.4 (Z)-5-(4-methoxybenzylidene- <i>d</i>)thiazolidine-2,4-dione 215.	148
6.9 Synthesis of (Z)-5-(4-propylbenzylidene)thiazolidine-2,4-dione.	149
6.9.1 4-Propylbenzaldehyde 216.	149
6.9.2 (Z)-5-(4-propylbenzylidene)thiazolidine-2,4-dione 182.	150
6.10 Synthesis of (Z)-5-(4-butylbenzylidene)thiazolidine-2,4-dione.	151
6.10.1 4-Butylbenzaldehyde 217.	151
6.10.2 (Z)-5-(4-butylbenzylidene)thiazolidine-2,4-dione 183.	152
6.11 Synthesis of (Z)-5-(4-<i>i</i>-propoxybenzylidene)thiazolidine-2,4-dione.	153
6.11.1 4- <i>i</i> -Propoxybenzaldehyde 218.	153
6.11.2 (Z)-5-(4- <i>i</i> -propoxybenzylidene)thiazolidine-2,4-dione 176.	154
6.12 Synthesis of (Z)-5-(4-<i>i</i>-butoxybenzylidene)thiazolidine-2,4-dione.	155
6.12.1 4- <i>i</i> -Butoxybenzaldehyde 219.	155
6.12.2 (Z)-5-(4- <i>i</i> -butoxybenzylidene)thiazolidine-2,4-dione 177.	156
6.13 Synthesis Of (Z)-5-(4-benzylbenzylidene)thiazolidine-2,4-dione.	157
6.13.1 4-Benzylbromobenzene 221.	157
6.13.2 4-Benzylbenzaldehyde 222.	158
6.13.3 (Z)-5-(4-benzylbenzylidene)thiazolidine-2,4-dione 184.	159
6.14 Synthesis Of (Z)-5-[(2<i>R</i>- and 2<i>S</i>-benzyl-3,4-dihydro-2<i>H</i>-1-benzopyran-6-yl)methylidene]thiazolidine-2,4-dione.	160
6.14.1 Chromone-2-carboxylic acid 225.	160
6.14.2 2,3-Dihydrobenzopyran-2-carboxylic acid 226.	161
6.14.3 2,3-Dihydrobenzopyran-2-carboxylic acid ethyl ester 227.	162
6.14.4 2,3-Dihydrobenzopyran-(2 <i>R</i>) and (2 <i>S</i>)-carboxylic acid ethyl ester, <i>R</i> - and <i>S</i> -227.	163
6.14.5 Increasing the Enantiomeric Excess of 2,3-Dihydrobenzopyran-(2 <i>S</i>)-carboxylic acid ethyl ester, <i>S</i> -227.	164
6.14.6 (2 <i>R</i>)- and (2 <i>S</i>)-(Hydroxymethyl)-2,3-dihydrobenzopyran, <i>R</i> - and <i>S</i> -228.	165
6.14.7 (2 <i>R</i>)- and (2 <i>S</i>)-Benzyl-2,3-dihydrobenzopyran, <i>R</i> - and <i>S</i> -229.	166
6.14.8 (2 <i>R</i>)- and (2 <i>S</i>)-Benzyl-6-(formyl)-2,3-dihydro-4 <i>H</i> -benzopyran, <i>R</i> - and <i>S</i> -230.	168
6.14.9 5-[(2 <i>R</i> - and 2 <i>S</i> -benzyl-3,4-dihydro-2 <i>H</i> -1-benzopyran-6-yl)methylidene]thiazolidine-2,4-dione, <i>R</i> - and <i>S</i> -231.	170
6.15 Synthesis of 5-[[5-benzoyl]methylidene]thiazolidine-2,4-dione.	171
6.15.1 2-Bromoacetophenone 232.	171
6.15.2 5-Formylsalicylaldehyde 233.	172
6.15.3 2-Benzoyl-5-benzofurancarboxaldehyde 234.	173
6.15.4 (Z)-5-[[5-benzoyl]methylidene]thiazolidine-2,4-dione 235.	174
6.16 Knoevenagel Condensation Between Cyclohexane Carboxaldehyde and Thiazolidine-2,4-dione.	175
6.16.1 (Z)-5-cyclohexanemethylidene thiazolidine-2,4-dione 236.	175

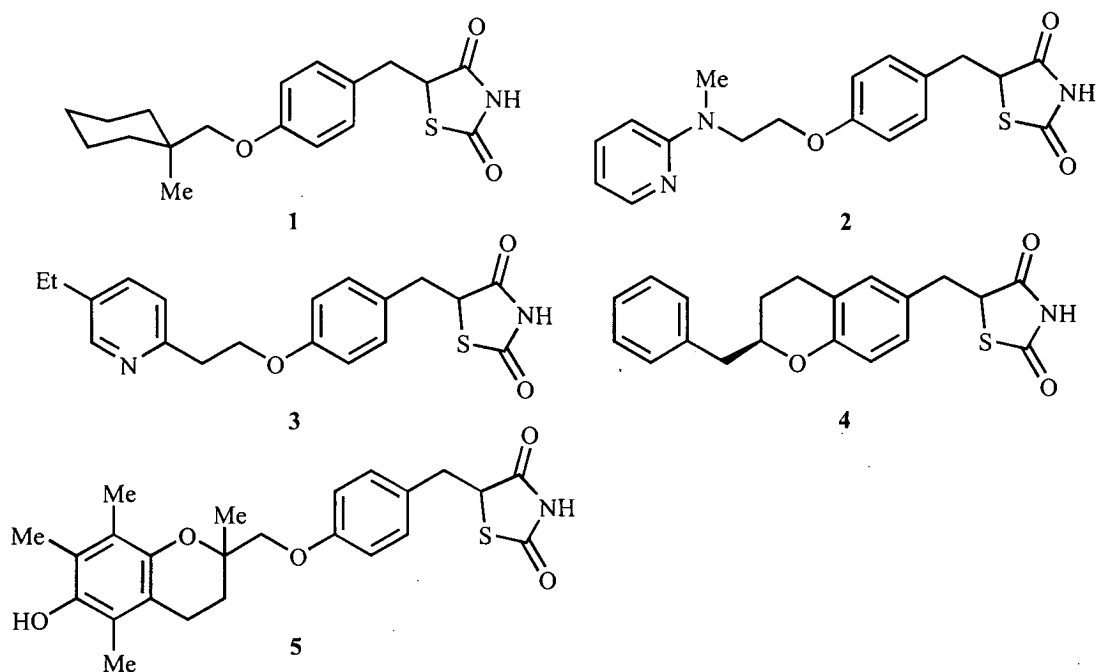
6.17 Synthesis Of (Z)-2-sulfanyl-3-(4-methoxyphenyl)propenoic acid Derivatives.	176
6.17.1 (Z)-2-sulfanyl-3-(4-methoxyphenyl)propenoic acid 237.	176
6.17.2 (Z)-2-methylsulfanyl-3-(4-methoxyphenyl)propenoic acid 238.	177
6.17.3 (Z)- 2-methylsulfanyl-3-(4-methoxyphenyl)propenoic acid methyl ester 239.	177
6.17.4 (Z)- 2-methylsulfanyl-3-(4-methoxyphenyl)propenoic acid ethyl ester 240.	178
6.17.5 (Z)-2-methylsulfanyl-3-(4-methoxyphenyl)propenoic acid amide 241.	179
6.18 Hydrogenation Of 5-Benzylidene 5-Membered Heterocycles.	180
6.18.1 General Procedures.	180
6.18.2 5-(4-{2-[methyl(2-pyridyl)amino]ethoxy} benzyl)thiazolidine-2,4-dione 2.	181
6.18.3 5-(4-methoxybenzyl)thiazolidine-2,4-dione 243.	182
6.18.4 5-(4-chlorobenzyl)thiazolidine-2,4-dione 244.	183
6.18.5 5-(4-cyanobenzyl)thiazolidine-2,4-dione 245.	183
6.18.6 5-cyclohexanemethylenethiazolidine-2,4-dione 247.	184
6.18.7 5-(4-methylbenzyl)thiazolidine-2,4-dione 249.	185
6.18.8 5-(4-ethylbenzyl)thiazolidine-2,4-dione 250.	185
6.18.9 5-(4-propylbenzyl)thiazolidine-2,4-dione 251.	186
6.18.10 5-(4-butylbenzyl)thiazolidine-2,4-dione 252.	187
6.18.11 5-(4-benzylbenzyl)thiazolidine-2,4-dione 253.	187
6.18.12 5-(4-methoxybenzyl)imidazolidine-2,4-dione 254.	188
6.18.13 5-(4-cyanobenzyl)imidazolidine-2,4-dione 255.	189
6.18.14 5-(2-methoxybenzyl)thiazolidine-2,4-dione 256.	189
6.18.15 5-(2,6-dimethoxybenzyl)thiazolidine-2,4-dione 257.	190
6.18.16 5-(3,5-di- <i>t</i> -butyl-4-hydroxybenzyl)thiazolidine-2,4-dione 258.	191
6.18.17 5-[2 <i>R</i> -benzyl-3,4-dihydro-2 <i>H</i> -1-benzopyran-6-yl)methyl]thiazolidine-2,4-dione 259.	191
6.18.18 5-(1-methyl-1-phenylmethyl)thiazolidine-2,4-dione 260.	192
6.18.19 5-(1-methyl-1-(4-methoxyphenyl)methyl)thiazolidine-2,4-dione 261.	193
6.18.20 5-(1-ethyl-1-(4-methoxyphenyl)methyl)thiazolidine-2,4-dione 262.	194
6.18.21 5-(4-methoxybenzyl- <i>d</i>)thiazolidine-2,4-dione 263.	195
7. EXPERIMENTAL : BIOTRANSFORMATIONS.	196
7.1 General Experimental.	196
7.2 Initial Optimisation Studies.	198
7.2.1 General Procedure.	198
7.2.2 Biotransformation Using Part of the Cell Suspension and 18.75 % v/v 1,4-Dioxane as Co-solvent.	198
7.2.3 Biotransformation Using all of the Cell Suspension and 18.75 % v/v 1,4-Dioxane as Co-solvent.	198
7.2.4 Biotransformation Using all of the Cell Suspension and 12 % v/v 1,4-Dioxane as Co-solvent.	199
7.2.5 Biotransformation Using all of the Cell Suspension and 7.5 % v/v DMSO as Co-solvent.	199
7.3 Biotransformations Using Varying Amounts of Co-solvent.	200
7.3.1 General Procedure.	200
7.3.2 Reduction of (Z)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione 172 with varying volumes of DMSO.	200
7.3.3 Reduction of (Z)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione 172 with varying volumes of 1,4-dioxane.	201
7.4 Biotransformations Using <i>Rhodotorula rubra</i> CBS 6469 to Afford Racemic Product.	202
7.4.1 General procedure.	202

7.4.2 5-(4-methoxybenzyl)thiazolidine-2,4-dione	243.	202
7.4.3 5-(4-chlorobenzyl)thiazolidine-2,4-dione	244.	203
7.4.4 5-(4-nitroobenzyl)thiazolidine-2,4-dione	246.	203
7.4.5 5-(4-cyanobenzyl)thiazolidine-2,4-dione	245.	204
7.4.6 5-(cyclohexylmethyl)thiazolidine-2,4-dione	247.	204
7.4.7 5-(4-methoxybenzyl- <i>d</i>)thiazolidine-2,4-dione	264.	205
7.5 Screen of Compounds against <i>Rhodotorula rubra</i> CBS 6469.		205
7.5.1 General Procedure.		205
7.5.2 Screen Results : Reduction of (<i>Z</i>)-5-(4-susbtitutedbenzylidene)thiazolidine-2,4-diones.		206
7.5.3 Screen Results : Reduction of (<i>Z</i>)-5-(4-susbtitutedbenzylidene)5-membered heterocycles.		207
7.5.4 Screen Results : Reduction of (<i>Z</i>)-5-(2-, 3-, di- and tri- substituted benzylidene)thiazolidine-2,4-diones.		207
7.5.5 Screen Results : Reduction of (<i>Z</i>)-5-(1-alkyl-1-(4-substitutedphenyl methylidene)thiazolidine-2,4-diones.		208
7.5.6 Screen Results : Reduction of (<i>Z</i>)-5-(4-methoxybenzylidene)-3-substituted thiazolidine-2,4-diones.		208
7.5.7 Screen Results : Reduction of Miscellaneous Thiazolidine-2,4-diones.		209
7.6 Immobilisation of <i>Rhodotorula rubra</i> CBS 6469.		210
7.6.1 Reduction of 172 by <i>Rhodotorula rubra</i> CBS 6469 Immobilised by Gel Entrapment with Calcium Alginate.		210
7.7 Mechanistic Studies.		211
7.7.1 Biotransformation of (<i>Z</i>)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione 172 in Deuterated Medium.		211
8. FUTURE WORK.		212.
9. APPENDIX 1 : HPLC DATA.		214
10. APPENDIX 2 : X-RAY CRYSTALLOGRAPHY DATA.		216
10.1 X-Ray Crystal Structure of 172 .		216
10.2 X-Ray Crystal Structure of 179 .		223
10.3 X-Ray Crystal Structure of 181 .		230
11. BIBLIOGRAPHY.		237

Background and Aims.

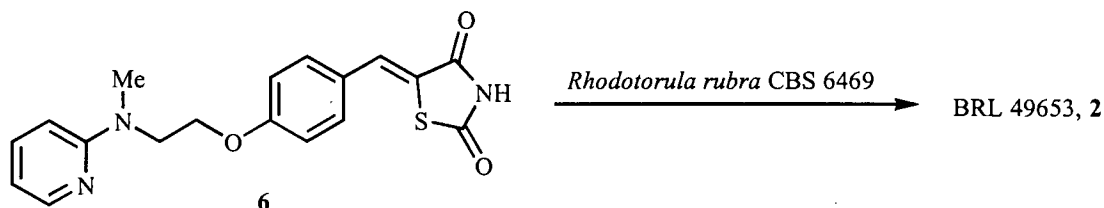
This project arose as a result of the pharmaceutical industry's interest in a new treatment for non-insulin dependent or type II diabetes mellitus (NIDDM). This therapeutical area has attracted attention from all the major companies over the last fifteen years, and has warranted an article in *Chemistry and Industry* earlier this year.¹

One class of compounds being investigated is based upon 5-benzyl thiazolidine-2,4-dione. The initial observation made in this area was the discovery of Ciglitazone **1** (Takeda) and its ability to alleviate the symptoms of NIDDM.² There have followed several related compounds : BRL 49653 **2** (SmithKline Beecham);³ Pioglitazone **3** (Takeda);⁴ Englitazone **4** (Pfizer);⁵ Troglitazone **5** (Sanyko, Parke Davis, Glaxo Wellcome).⁶

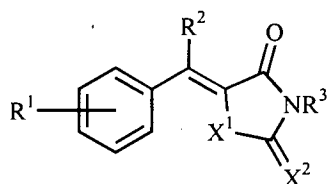


The final step in the synthesis of all these compounds is the reduction of the appropriate benzylidene compound. In the case of BRL 49653 and its analogues

SmithKline Beecham found this to be problematic. This led to the development of a microbial procedure, using *Rhodotorula rubra* CBS 6469, to reduce the double bond of **6** which has subsequently been modified for the synthesis of **2** on pilot plant scale.^{7,8}



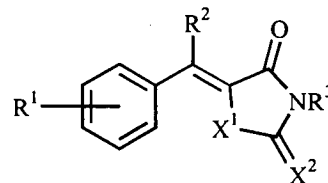
The initial aims of this project were two-fold. Firstly the synthesis of a range of compounds, based on the template shown below, was proposed to ascertain which structural features were required for a compound to be accepted as a substrate by the reductase system. In doing so it was envisaged that a range of relative reaction rates could be measured.



The second aspect of the project aimed to elucidate the mechanism of reduction by the introduction of isotopically labelled substrates.

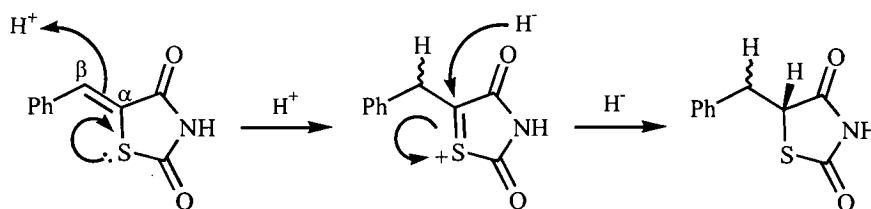
Abstract.

A range of compounds designed around a template based upon (Z)-5-benzylidene thiazolidine-2,4-dione were synthesised by Knoevenagel condensation. These were screened against *Rhodotorula rubra* CBS 6469, a red yeast, using a modification of a process developed by SmithKline Beecham.¹ This has allowed the construction of a 'map' defining the structural features of the template which are required to afford reduction of the carbon-carbon double bond. It has been shown that X¹ and X² must be S and O respectively whilst R³ can be methyl or benzyl. Alkyl substitution at R² is not tolerated but a variety of substituents can be present at R¹. *Para* groups can be accommodated at R¹, but only methyl in the alkyl series, as well as the *meta* methoxy group. It has also been shown that replacement of the phenyl ring with a cyclohexyl moiety still allows reduction to take place.



Relative reaction rates are also discussed along with the development of a process to afford reduction using *Rhodotorula rubra* CBS 6469 immobilised in calcium alginate beads.

By carrying out reductions on deuterated compounds, and in deuterated medium, steps have been taken towards the elucidation of the mechanism of reduction. The following scheme is proposed as a possible mechanistic pathway.



1. B.C.C. Cantello, D.S. Eggleston, D. Haigh, R.C. Haltiwanger, C.M. Heath, R.M. Hindley, K.R. Jennings, J.T. Sime and S.R. Woroniecki, *J. Chem. Soc. Perkin Trans. 1*, 1994, 3319.

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Thanks goes to Dr. Mike Urquhart and Latifa Youssef at SmithKline Beecham, Tonbridge for supplying samples of **6** and **248** whilst Kaye Cattanagh deserves a special mention for her assistance in developing a chiral hplc method.

Finally I would like to thank my parents and Alison for all their support, both emotional and financial, without which completion of this degree would surely have been an impossibility.

Abbreviations.

There follows a list of common abbreviations used in the text of this thesis.

Ac	Acetyl
bp	Boiling point
δ_{H}	Chemical shift of proton (nmr)
δ_{C}	Chemical shift of carbon (nmr)
d	Day
DCE	Dichloroethane
DCM	Dichloromethane
d.e.	Diastereomeric excess
dec.	Decomposes
DEPT	Distortionless enhancement through polarisation transfer
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
e.e.	Enantiomeric excess
EI	Electron impact ionisation
gc	Gas chromatography
h	Hour
hfc	3-(heptafluoropropylhydroxymethylene)-(+)-camphorate
HMTA	Hexamethylenetetramine
hplc	High performance liquid chromatography
IBX	2-Iodoxybenzoic acid
J	Spin coupling constant
min	Minute
mol%	Molar percentage
mp	melting point
m/z	Mass to charge ratio

NAD ⁺	Nicotinamide adenine dinucleotide (oxidised form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADP ⁺	Nicotinamide adenine dinucleotide phosphate (oxidised form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
n.d.	Not determined
NIDDM	Non-insulin dependent diabetes mellitus
nmr	Nuclear magnetic resonance
Pd/C	Dry palladium on activated charcoal
Ph	Phenyl
ppm	Parts per million
Py	Pyridyl
R _F	Retention factor (tlc)
R _T	Retention time (hplc/chiral gc)
sat.	Saturated
s.g.	Specific gravity
spp.	Species
TBDMS	Tertiary butyl dimethyl silyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
tlc	Thin layer chromatography
Tris	Tris(hydroxymethyl)aminomethane
w/v	Weight to volume ratio
v/v	Volume to volume ratio
YADH	Yeast alcohol dehydrogenase

1. Introduction.

A modern biotransformation involves the harnessing of an enzymatic system, whether as an isolated entity or as part of a whole cell micro-organism, to afford a chemical transformation usually with high regio, chemo and stereoselectivity and can be a powerful tool to a synthetic organic chemist. This introductory chapter intends to give a brief history of how this tool emerged, followed by the associated advantages, and disadvantages over standard chemical methods, before discussing the range of reduction reactions catalysed by such systems.

1.1 Introduction To Biotransformations.⁹⁻¹⁴

It is widely accepted that the origins of the modern biotransformation are to be found in the metabolism of sugar to ethanol by yeast. This began in earnest with the award of a patent to Coffey in 1833 for the production of ethanol from fermented grain wort. Three years later Payen and Persoz extracted diastase from malted barley which was used to separate and break down starch for use in the fermentation process. In 1838 this use of diastase prompted Berzelius to introduce the term 'catalysis' and write :

'One can hardly assume that this catalytic process is the only one in the vegetable kingdom. On the contrary, it gives reason to believe that within living plants and animals thousands of catalytic processes are going on between the tissues and the fluids, producing a multitude of chemical compounds, the creation of which out of the common raw material, the sap of plants or blood, has up to now been unexplained, and which may possibly be found in the future to depend on the catalytic power of the living tissues'

Since these original observations over a century and a half ago the concept has flourished. Although the production of ethanol had all but given way to synthetic processes based upon petroleum the changing social climate in recent years has led to an increase in the use of the fermentation process.

Many other fermentation processes still remain and serve us well. In 1934 Reichstein introduced a procedure utilising *Acetobacter xylinum* for the synthesis of ascorbic acid, a process which is still the basis of modern manufacturing procedures. Lactic acid formation from lactose using *Lactobacillus bulgaricus* is carried out today, based on a process introduced in 1880. Amino acids can be, and are, produced by fermentation. For example L-glutamate is produced from starch or molasses by *Corynebacterium glutamicum*. Arguably the most important breakthrough in modern medicine was the introduction of penicillins. Both the penicillins and cephalosporins are secondary metabolites produced by the fungi *Penicillium* spp. and *Cephalosporium* spp. and have led to the development of 'semi-synthetic' processes.

The reason these fermentation techniques have survived is their efficiency. They can produce complex molecules with excellent stereoselectivity more easily, and with less expense, than the alternatives offered by synthetic organic chemistry.

1.2 Advantages and Disadvantages of Biocatalysis.^{9-12,15,16}

It is this stereoselectivity which is the main advantage of biocatalysis. The introduction of regulations surrounding the use of racemic mixtures as pharmaceuticals has led to an increase in technologies designed to produce compounds with high stereoselectivity. One method of achieving this is the use of compounds from the 'chiral pool' as starting materials in a synthetic route, although there are limitations on the availability of appropriate compounds. Direct resolution offers a means for the separation of a racemate into the constituent isomers. Although this offers the advantage of producing both enantiomers, the maximum yield available of each is 50 %. Theoretically, the use of chiral auxiliaries allows the production of a quantitative yield but increases the number of steps involved in a synthetic route as the auxiliary needs to be attached before transformation and removed afterwards. This is not a factor with chiral catalysts where the catalyst imparts stereoselectivity through a transient interaction with the reagent, however

they are usually complex molecules requiring complex synthesis by one of the above methods.

The inherent chirality of enzymes, imparted by the presence of L-amino acids, allows the 'recognition' of any chirality present in the substrate. As a result, different enantiomers of a substrate can react at different rates affording a kinetic resolution. This also allows the formation of an optically active product from a prochiral compound. Although this constitutes an enzyme's main advantage for an organic chemist it can also be a major disadvantage as it is not always possible to obtain the other enantiomer, as is possible with a synthetic chiral catalyst, as it is not always possible to switch the inherent chiral induction of a particular enzymatic system.

Enzymes can also exhibit high levels of chemo and regioselectivity. As an enzyme's purpose is to act on a single functional group it can do so without affecting other functionality in the molecule that may react under chemical conditions. It is also possible for an enzyme to act upon one functional group whilst leaving a similar group in a different environment untouched.

Enzymes also act under mild conditions, typically physiological conditions, such as neutral pH and temperatures around 30 °C. This helps to minimise side reactions that may occur under more extreme conditions. However, this can be a disadvantage. If the catalytic process is slow under these conditions there is little scope for alteration of the reaction conditions, as extreme pH and elevated temperatures can lead to deactivation of the enzyme.

One serious drawback is that enzymes exhibit highest activity in water, whereas the majority of organic compounds are only sparingly soluble in aqueous systems. This has led to extensive study centred on the use of biotransformations in organic solvents. When water can be used there are obvious advantages in terms of cost and the disposal of waste. Enzymes and cells are themselves environmentally acceptable as they are completely biodegradable.

Enzymes are extremely efficient catalysts with acceptable rates being observed with 10^{-3} - 10^{-4} mol% of the enzyme. Contrast this with the typical 0.1-1 mol% utilised in reactions with chemical catalysts and it is evident that the enzyme is a much more efficient catalyst.

One problem associated with the use of enzymes is their capability to produce an allergic reaction, also certain organisms are pathogenic. However, most chemicals have an associated hazard and, as with chemicals, careful handling of any enzyme minimises the risk to the user.

1.3 Using Biocatalysis : Whole Cell Systems or Isolated Enzymes ? ^{9-11,16,17}

Fermentation processes are the life processes of the organism. They involve growing the cells with a natural source of elements to produce only natural products of metabolism. These processes can be lengthy and make use of many enzymatic steps within the cell but can produce many by-products making isolation of the required product tedious. They also require large biomass and only produce natural products.

The majority of modern microbial biotransformations involve harnessing the use of a few enzymatic steps to afford a catalytic transformation, such as a carbonyl reduction or the hydrolysis of an ester, of either a natural or non-natural substrate. These reactions produce few by-products making the isolation of the desired material easier. This process is sometimes referred to as 'enzymation' and allows the use of a microbial system to carry out one 'synthetic step'.

An organic chemist may find the idea of using a whole cell system to carry out a biotransformation intimidating. This is unsurprising as a certain degree of expertise is required in the growth and handling of these organisms. However, the use of lyophilised baker's yeast greatly simplifies the process. Indeed Veschambre *et al.*,

have described 'a four hour experiment for undergraduate students' involving the baker's yeast reduction of α -diketones.¹⁸

The use of whole cells can have several advantages, mainly that the addition of co-factors and a suitable recycling system is unnecessary. However, expensive equipment can be required, work-up is often tedious due to the large volumes involved and low concentration tolerance may lead to low productivity. As there are many other enzymes present in the system there is always the possibility of side reactions occurring. The use of immobilised cells, for example by entrapment in a polymer matrix, can allow the re-use of cells but lower activities are often observed.

Reactions using purified, or partially purified, enzyme preparations can involve simpler apparatus, simpler work-up, and due to higher concentration tolerance may show better productivity. Highest activities are exhibited when used in water but lipophilic substrates are insoluble and work-up involves extraction. When using an organic solvent, again, they are easy to perform and lipophilic substrates are soluble but can exhibit lower activities. Immobilised enzymes make enzyme recovery easier but activity can be lost during immobilisation.

1.4 Classes of Enzyme.⁹⁻¹¹

Enzymes are classified by the type of reaction they catalyse and are categorised into six groups. The group number constitutes the first of a four figure Enzyme Commission (EC) identification number associated with each enzyme.

Group 1 consists of the oxidoreductase enzymes which are capable of interconverting alcohols with aldehydes/ketones, alkenes with alkanes and oxygenating C-H bonds. Baeyer-Villiger reactions are also possible.¹⁹⁻²² The majority are carried out using whole cells, as they require a co-factor, particularly baker's yeast (*Saccharomyces*

cerevisiae) due to its availability and ease of use, and there are a wide range of dehydrogenases and oxygenases available.

The second group is made up by the transferase enzymes which catalyse the transfer of a functional group (aldehydic, ketonic, acyl, sugar, phosphoryl or methyl) between molecules. This class of enzymes includes transketolase (EC 2.2.1.1) which can be used to extend the chain of a variety of aldoses stereospecifically by two carbon units and is appearing to be a promising catalyst in organic synthesis.²³⁻²⁹

Hydrolases, the most commonly used enzymes in organic synthesis, make up group three. These enzymes are capable of hydrolysing esters, amides, lactones, lactams, epoxides, nitriles, anhydrides and glycosides. This class of enzymes include lipases, the natural role of which is the hydrolysis of triacylglycerol into glycerol and fatty acids. However, lipases are readily available, inexpensive and can have a broad substrate range. This has led to them being the most studied enzymes in organic synthesis.

Group 4 consists of lyases, enzymes capable of catalysing the addition-elimination of small molecules on C=C, C=O and C=N bonds. Indeed *Rhodotorula rubra* has been shown to catalyse the amination of the cinnamic acid double bond to produce optically pure *S*-amino acids.³⁰

The last two groups are of little use in organic synthesis. Isomerases (group 5) catalyse reactions such as racemisation and epimerisation and include mutarotase (EC 5.1.3.3) which converts α -D-glucose to β -D-glucose. Ligases make up group 6 and catalyse the formation-cleavage of C-O, C-S, C-N and C-C bonds with concomitant triphosphate hydrolysis.

1.5 Reduction of Carbonyl Groups.^{9-12,15-17,31-34}

Alcohol dehydrogenases are enzymes that catalyse the oxidation of alcohols and the reduction of aldehydes and ketones. These enzymes are dependent on NAD(P)H for the reduction of carbonyl groups by delivery of hydride from the reduced form of the pyridyl co-factor.

The majority of the alcohol dehydrogenase systems that have been studied are baker's yeast and horse liver alcohol dehydrogenase (HLADH), although others have been utilised. There are several general rules that can be applied to these reduction reactions. The first regards the size of the substrates accepted by various different alcohol dehydrogenases (Fig. 1-1).^{9,31,35} While micro-organisms usually show a broad range of substrate specificity the range associated with the isolated enzymes are generally narrower. Indeed, yeast alcohol dehydrogenases (YADH) only reduce aldehydes and short chain ketones whilst *Thermoanaerobium brockii* alcohol dehydrogenase (TADH) is mainly specific for methyl or ethyl ketones. Alkyl substituted cyclic aliphatic ketones are reduced by HLADH whilst *Pseudomonas testosteroni* alcohol dehydrogenases (PTADH) displays a wider specificity towards polycyclic compounds. Different hydroxy steroid dehydrogenases (HSDH) recognise a variety of carbonyl compounds around the steroid template.

The second rule surrounds the stereochemistry of the resulting alcohol and is governed by Prelog's rule.³⁶ This rule was initially based upon the reduction of carbonyl groups by *Curvularia falcata* and defines the groups either side of the carbonyl as small and large. If the carbonyl derivative is represented with the smaller group to the right the rule states that hydride delivery will occur from the rear (Fig. 1-2).

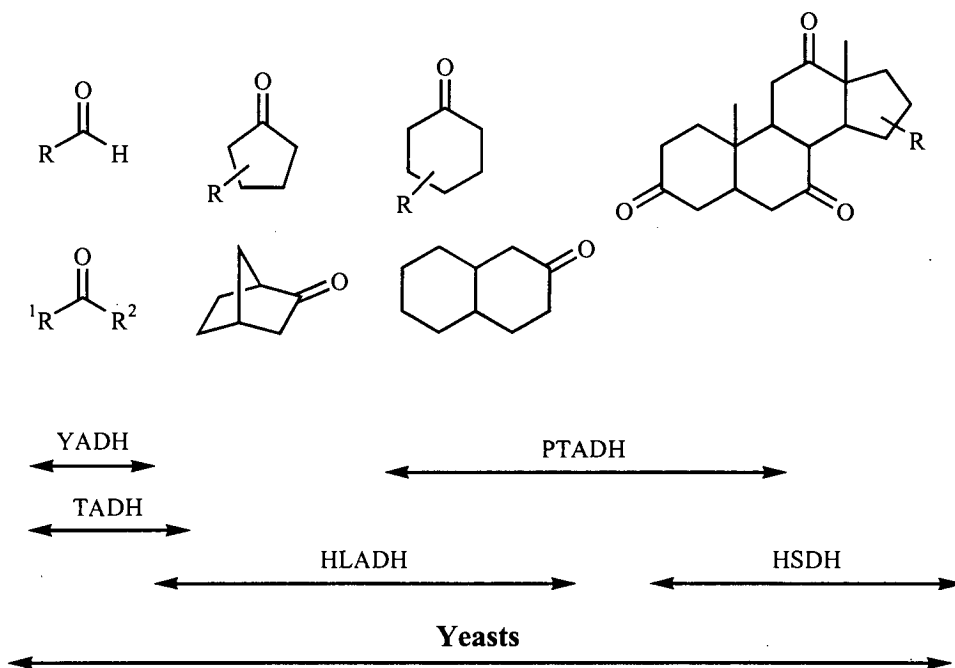


Fig. 1-1 : Relationship Between Various Alcohol Dehydrogenases and Substrate Size.

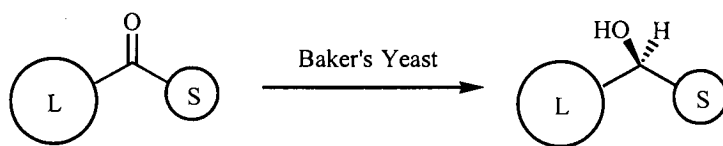


Fig. 1-2 : Diagrammatic Representation of Prelog's Rule.

Although not all dehydrogenases follow this rule it is constantly used to define the mode of hydride addition displayed by each dehydrogenase. In addition to baker's yeast, both HLADH and TADH adhere to Prelog's rule, as do hydroxysteroid dehydrogenases. On the other hand *Pseudomonas* spp., *Mucor javanicus*, *Lactobacillus kefir* and *Pichia farinosa* alcohol dehydrogenases do not.^{9,31,37}

Rhodotorula rubra has been shown to perform several carbonyl reductions and one such instance, reported in 1993 by Yamazaki and Kobayashi, related to the growing interest regarding the application of enzymes in organic synthesis to organometallic chemistry, and focused upon the reduction of (trifluoroacetyl)ferrocene.³⁸ A study on the reduction of acetophenone and the mono, di, and tri α -fluorinated analogues showed that the reduction of acetophenone favoured reduction in the Prelog fashion

in 98 % e.e.. However, as the number of fluorine atoms increased the e.e. fell until the anti-Prelog product was formed in 40 % e.e. in the reduction of trifluoroacetophenone. The introduction of fluorine atoms also increased the rate of conversion. The cell free extract of *Rhodotorula rubra* IFO 899 yielded two fractions which showed activity towards the reduction of (trifluoroacetyl)ferrocene although with opposing stereoselectivities.

In the previous year Sicsic showed that *Rhodotorula rubra* ATCC 4056 was capable of reducing the carbonyl of both α - and β -tetralones to the corresponding tetralols with good stereoselectivity.³⁹ The production of optically pure tetralols is of importance due to their use as chiral building blocks in organic synthesis.

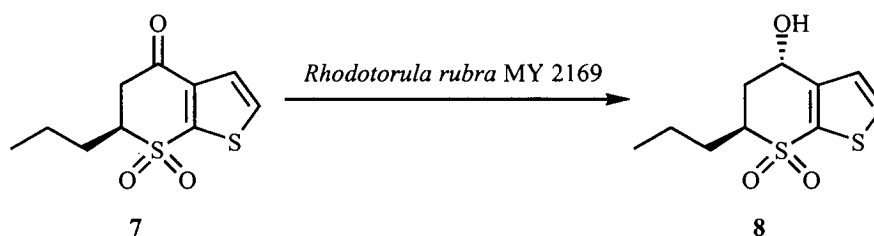


Fig. 1-3 : *Rhodotorula rubra* Mediated Reduction of a Ketosulfone.

In addition it has been shown that *Rhodotorula rubra* MY 2169 is capable of reducing the ketosulfone **7** to the *trans*-hydroxysulfone **8** in > 91 % d.e. (Fig. 1-3).⁴⁰

1.6 Reduction of Carbon-Carbon Double Bonds.^{9-12,15,16,31-33,41}

1.6.1 General Points.

The enantiotopic selective reduction of prochiral carbon-carbon double bonds has been studied using a variety of organisms. The enzymes involved are NAD(P)H-dependent enoate reductases which can be found in organisms such as *Clostridium* spp., *Beauveria* spp. and baker's yeast. Although several enzymes responsible for the reduction have been isolated, the majority of reactions are carried out with whole cells of baker's yeast, mainly due to problems of cofactor recycling and the sensitivity of the enoate reductases towards oxygen.

To be effectively reduced by an enoate reductase the double bond has to be 'activated' by an electron withdrawing substituent, either a carbonyl or nitro group. Due to the susceptibility of carbonyl reduction by the numerous dehydrogenases in baker's yeast it is unsurprising that, when the double bond is activated by a carbonyl group, the alcohol can be readily formed as a side product.

A variety of different functionalities can be present on the double bond and each class of compound will be dealt with in turn with the main focus on yeast reductions.

1.6.2 Yeast Reductions.

1.6.2.1 Reduction of Cinnamaldehydes and Cinnamyl Alcohols.

In 1975, through a series of deuterated studies, Fuganti determined the steric course of saturation of the carbon-carbon double bond of cinnamyl alcohol and cinnamaldehyde with baker's yeast.⁴²

Reduction of the deuterated aldehyde **9** was found to produce the 1*S* alcohol **10** by the introduction of a *pro-R* hydrogen atom (Fig. 1-4). A similar experiment using purified YADH, NAD⁺ and ethanol reduced the aldehyde in the same manner but left the olefinic bond unaffected.

Reduction of the two deuterated olefins **11** and **13** formed 2*S*-**12**, by introduction of a *pro-R* hydrogen, and 3*R*-**14** respectively. Reduction of **15** to **16** confirmed these observations and also showed that exchange of deuterium for a *pro-R* hydrogen atom takes place at position 1. This is in agreement with the stereochemical course of carbonyl reduction in **9** as the alcohol would be reduced *via* the aldehyde.

This reaction is still used as a source of optically active 3-phenyl-2-methyl-1-propanols and has been extended to the reduction of **17** and **19**.^{43,44} Fuganti has shown that **17** can be reduced to produce **18** which is used in the synthesis of α -tocopherol, however **19** gives rise to a mixture of **20** and **21** (Fig. 1-5).⁴⁴

Later it was noted that, as well as the carbonyl and carbon-carbon double bond reduction of cinnamaldehyde, baker's yeast was capable of producing a diol formed from the aldehyde and a C₂ unit (Fig. 1-6).⁴⁵ For example, reduction of 2-bromo cinnamaldehyde **22** produces 10 % of **23**, 5 % of **24** and 50 % of **25**, a fact that has been exploited in the synthesis of L-amectose, D-(-)-*allo*-muscarine, L-olivomycose, *N*-trifluoroacetyl-L-acosamine, 4-hexanolide, derivatives of 2,3,6-trideoxy-3-*C*-methyl-3-aminohexose and (-)-frontalin.⁴⁶⁻⁵² It has also been shown that this is possible with α -methyl- β -(2-furyl)acrolein.⁵³

More recently Šunjić reported a study of reduction of a series of *para* derivatives of 2-methyl cinnamaldehyde **26** by baker's yeast with a high degree of enantioselectivity at the α carbon (Fig. 1-7).^{54,55} This is in agreement with Fuganti's mechanism which demonstrates enantioselective hydrogen incorporation at either end of the double bond (Fig. 1-4).

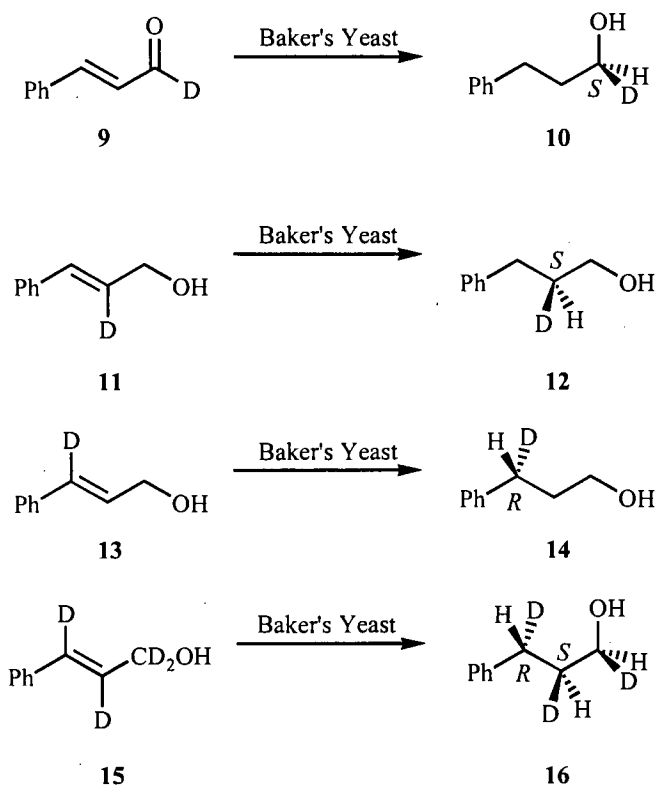


Fig. 1-4 : Determination of the Stereochemical Course of Baker's Yeast Reduction of Cinnamyl Alcohol and Cinnamaldehyde.

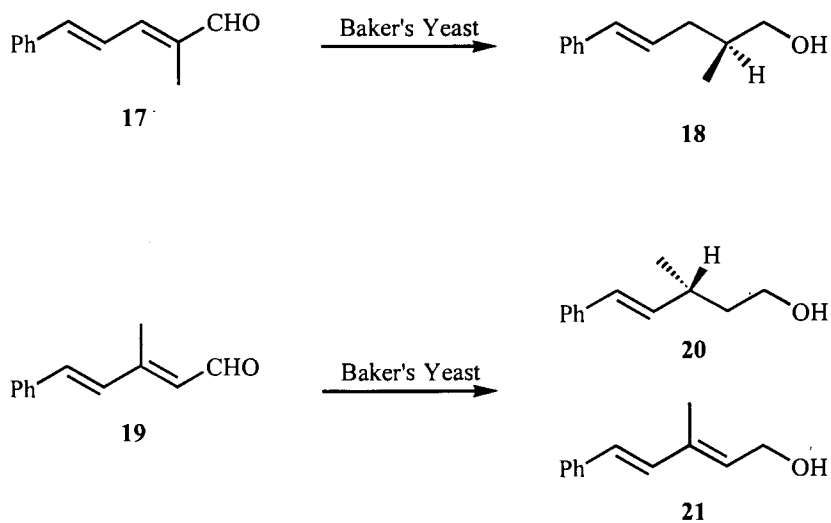


Fig. 1-5 : Baker's Yeast Reduction of 5-phenyl-penta-2,4-dienes.

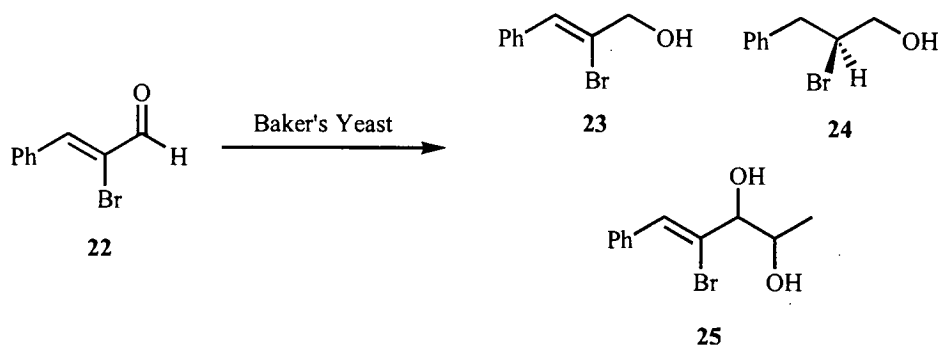


Fig. 1-6 : Baker's Yeast Reduction of α -Bromocinnamaldehyde **22**.

Reduction of both the carbonyl and the carbon-carbon double bond are reported but the saturated aldehyde, often proposed as an intermediate, was not detected. However, it was noted that delivery of saturated aldehydes to the system led to carbonyl reduction and it was proposed that they were present in very low, steady state, concentrations.

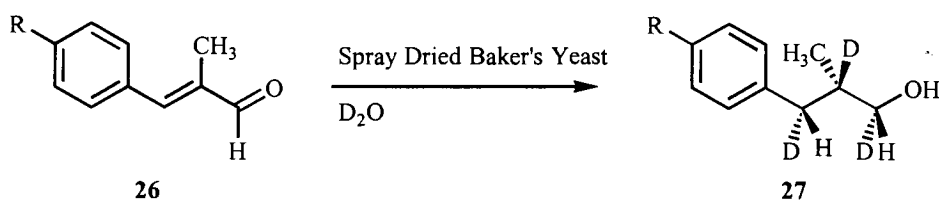


Fig. 1-7 : Baker's Yeast Reduction of **26** in D_2O .

It was shown that the rate of reduction of the carbonyl is dependent on the nature of the *para* substituent, with an electron withdrawing group enhancing the rate of reduction whilst an electron donating group decreased the rate. No such correlation was noted for the reduction of the carbon-carbon double bond.

The reduction of 2-methyl cinnamaldehyde **26** with spray dried yeast in D_2O resulted in trideuterated **27** which was indicative of incorporation of deuterium into the cofactor then delivery to the substrate.

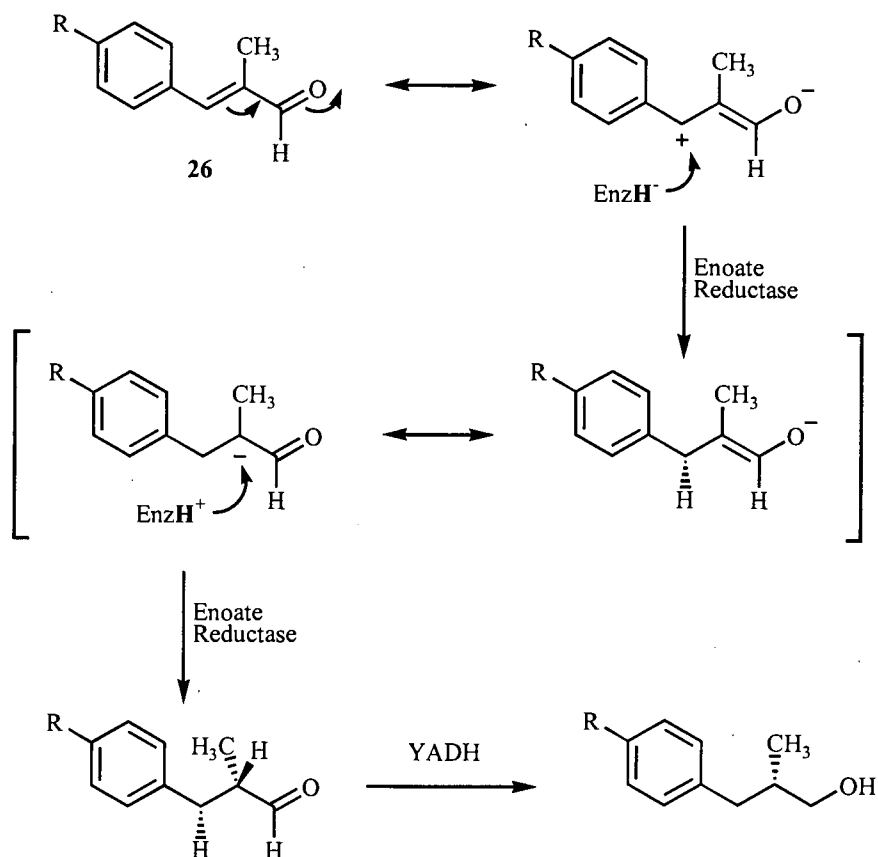


Fig. 1-8 : Proposed Mechanism for Baker's Yeast Reduction of 26.

Generally it is proposed that 'attack of hydrogen' occurs without specifying the anionic or cationic nature of this hydrogen. However, Fuganti and Servi have proposed formal hydride delivery to the β carbon in the reduction of α,β unsaturated lactones (Sect. 1.6.2.6).^{56,57} Šunjić postulates that if this was the case here then the proton transfer is enantioselective and was probably delivered by the enzyme. Enzymatic protonation at the emerging chiral centre was recently proposed for the lipase catalysed hydrolysis of prochiral enolacetates.^{58,59}

Also if it was attempted to reduce a double bond chemically by addition of a proton first it would involve the use of a strong acid and a hydride donor.⁶⁰ If this was to be achieved biochemically it would involve the delivery of a strongly acidic proton from the active site to the substrate. At the time this was unknown but Smallridge has

since reported a mechanism for the reduction of β -nitrostyrenes that involves protonation as the first step.⁶¹

The accumulation of these facts prompted Šunjic to propose the mechanism outlined in Fig. 1-8 as the likely mechanism for the reduction of **26**.

In a study on the reduction of cinnamyl alcohols by baker's yeast Gramatica concluded that the alcohol is oxidised to the aldehyde before double bond reduction occurs.⁶² It was noted that although the *E* alcohols were reduced the *Z* isomer was not. Also 2-methyl and 2-ethyl cinnamyl alcohols were reduced whereas the 3-methyl was not.

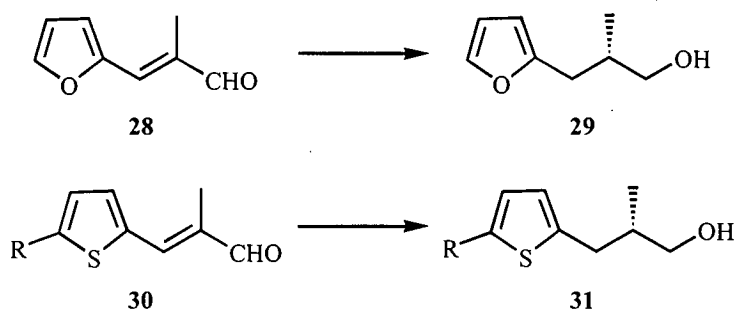


Fig. 1-9: Baker's Yeast Reduction **28** and **30**.

Compounds produced by replacement of the phenyl ring of cinnamaldehyde by various heterocycles can also be reduced using baker's yeast. For example, a collaboration between Fuganti and colleagues with Hogberg has demonstrated the reduction of **28** and **30** to **29** and **31** respectively (Fig. 1-9).^{63,64} It can be seen that the resultant stereochemistry is in agreement with that previously noted (Figs. 1-4 and 1-7). It was also noted that reduction of **28** involves a slow carbon-carbon double bond reduction followed by a fast carbonyl reduction.⁶⁴

The formation of **29** and **31** in this manner has been utilised in a synthesis of a pine sawfly sex pheromone.⁶⁵

1.6.2.2 Reduction of α,β -Unsaturated Ketones.

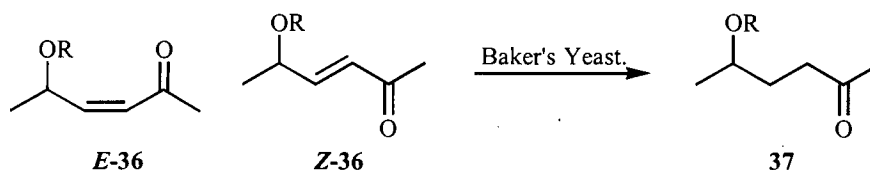
Kawai has shown the ability of baker's yeast to reduce the double bonds of a range of 4-phenylbutenoates **32** to produce mixtures of **33**, **34** and **35**, a phenomenon previously noted by Fuganti.^{66,67} It is interesting to note that the substitution on the ring influences both the extent of reduction and e.e. of the products (Fig 1-10). For example introducing a *para* hydroxyl on the phenyl ring retards reduction whilst introduction of *meta* methoxy group improves both the yield and the e.e.. Heteroaromatic rings are also accepted with the pyridyl ring affording racemic **32g**; however, when a furyl ring is incorporated the major product is the unsaturated alcohol **34h**.

Later studies suggested that only one enzyme was involved as reduction of various concentrations of **32a** produced **33a** with approximately 75 % e.e..⁶⁸ It was also noted that reduction occurs *via* formal *trans* addition across the double bond even when racemic product is formed, indicating that the lack of stereoselectivity is due to random positioning in the active site rather than random stereochemistry of reduction.

Kawai also looked at the reaction pathway and has shown that double bond reduction can only occur if it precedes carbonyl reduction.⁶⁸ This was noted in the reduction of **28** where the initial reaction is a slow double-bond reduction followed by a fast carbonyl reduction.⁶⁴ This ties in with the fact that only activated double-bonds can be reduced.

It has been shown that the baker's yeast reduction of *E*- and *Z*-**36** results in the formation of **37** (Fig. 1-11).⁶⁹ Initially it appeared that the reaction, at low conversion, proceeded with kinetic resolution as samples of **37** were enantiomerically enriched, however *Z*-**36** and *E*-**36** gave rise to *R*-**37** and *S*-**37** respectively.

	32	33	34	35	
	Ar	Time (h)	Yield/e.e. (%)		
a	Ph	30	42/71	3	9
b	4-HO-Ph	76	20/58	0	0
c	2-MeO-Ph	48	13/>95	0	0
d	3-MeO-Ph	48	72/>95	0	7
e	4-MeO-Ph	20	73/61	0	16
f	3,4-di-MeO-Ph	60	59/>95	1	6
g	2-Py	50	59/1	7	23
h	2-Furyl	80	5/67	30/>99	13
i	5-Me-2-Furyl	24	8	0	0

Fig. 1-10 : Baker's Yeast Reduction of **32**.Fig. 1-11 : Baker's Yeast Reduction of α,β -Unsaturated Methyl Ketones.

Deuteration studies showed that, in the reduction of both *E*- and *Z*-**36**, enzymic saturation of the double bond occurred in an *anti* fashion with formal hydrogen delivery at position β to the carbonyl group from the *re* face. They also showed that the reaction does proceed with kinetic resolution, with the enantiomeric preference varying from *R* to *S* in going from *Z* to *E*.

The cyclic α,β -unsaturated diketone **38** can also be reduced by baker's yeast as shown by Leuenberger *et al.*, in the synthesis of carotenoids.⁷⁰ An 80 % yield of dione **39** was obtained on a 13 kg scale with the production of minor products **40** (6 %) and **41** (7 %) as a result of the reduction of the 1- and 6-keto moiety respectively (Fig. 1-12).

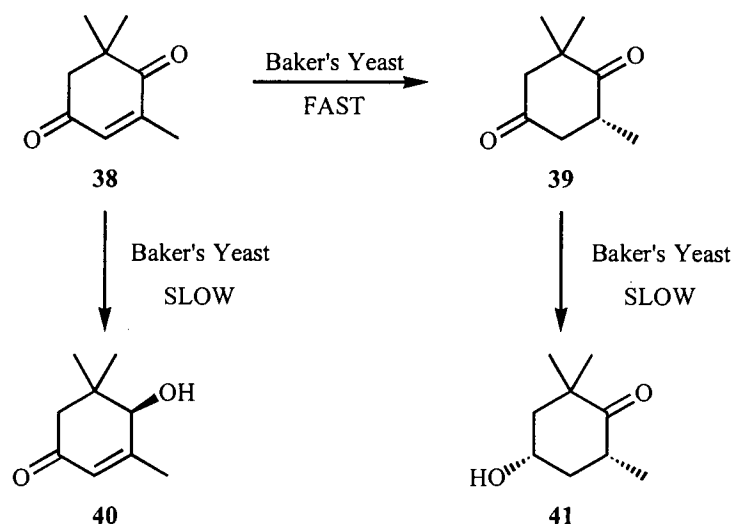


Fig. 1-12 : Baker's Yeast Reduction of a Cyclic α,β -Unsaturated Diketones.

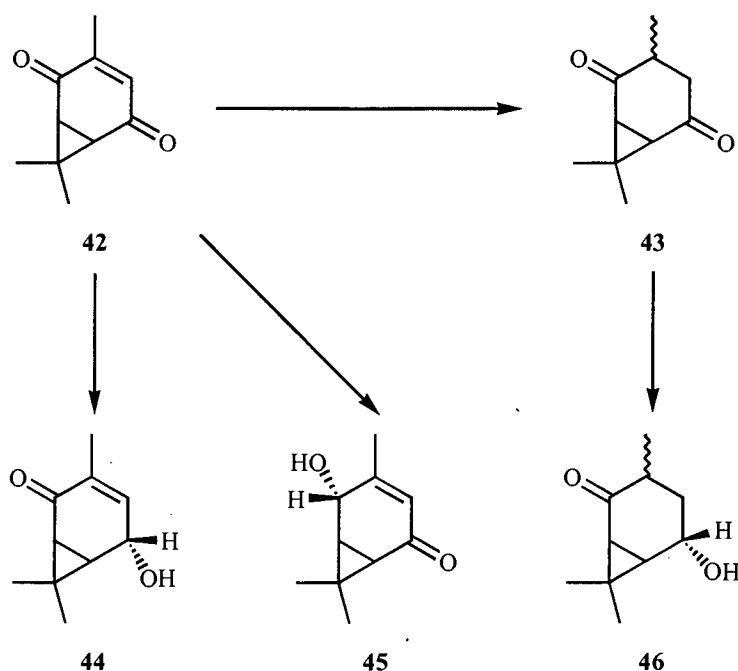


Fig. 1-13 : *Rhodotorula mucilangosa* Reduction of 3-Caren-2,5-dione **42**..

Similarly *Rhodotorula mucilaginosa* has been shown to reduce the carbon-carbon double bond of 3-caren-2,5-dione **42** (Fig. 1-13).⁷¹ However, in this instance the main products are (+)-*trans*-3-caren-5-on-2-ol **45** and (+)-2-caranon-2-ol **46**.

It has also been reported that yeast is capable of reducing the Woodward lactone **47** (Fig. 1-14).¹⁶

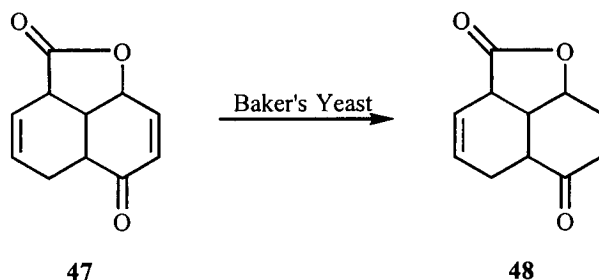


Fig. 1-14 : Baker's Yeast Reduction of Woodward's Lactone.

Sakai and Utaka have shown that baker's yeast can reduce the terminal double bond of 3-alkyl-3-buten-2-ones **49a-e** and 3-phenyl-3-buten-2-one **49f** (Fig. 1-15).⁷² In all cases the reduction proceeds in accordance with Prelog's rule to produce *R*-**50** in varying yields depending on the alkyl chain length. It can be seen that no reaction occurs with **49a** and **49e** with the maximum yield exhibited with **49c**.

Reduction of a terminal double bond is implicated in the formation of propiophenone **55** in the reduction of 3-chloropropiophenone **52**. It has been shown that this occurs *via* formation of the vinyl ketone **54** followed by reduction of the carbon-carbon double bond to produce **55** (Fig. 1-16).⁷³

		$\xrightarrow{\text{Fermenting Baker's Yeast}}$				
	49			50	51	
	R	Time (h)	Recovery of 49 (%)	Yield of 50 (%)	e.e. of 50 (%)	Yield of 51 (%)
a	C ₅ H ₁₁	87	0	0	-----	0
b	C ₆ H ₁₃	64	0	24	> 99	1
c	C ₈ H ₁₇	22	0	29	> 98	6
d	C ₁₁ H ₂₃	90	20	22	> 98	0
e	C ₁₂ H ₂₅	74	78	0	-----	0
f	Ph	60	0	24	95	6

Fig. 1-15 : Fermenting Baker's Yeast Reduction of 3-alkyl and 3-phenyl-3-buten-2-ones.

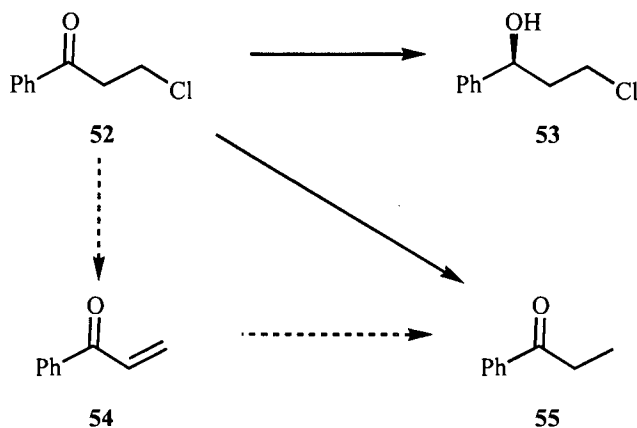


Fig. 1-16 : Formation of Propiophenone **55** on Reduction of 3-Chloropropiophenone **52** with Baker's Yeast.

Similarly the β -hydroxy α -methylene ketones **56** were reduced in good yield and moderate to high e.e. to produce *syn*-**57** (3*R*,4*S*) and *anti*-**57** (3*R*,4*R*) (Fig. 1-17).⁷⁴ Those with the shorter R group were reduced more quickly and the e.e of *syn*-**57** is always good; however, as the chain length increases *anti*-**57** becomes more favoured and the e.e. of the *anti* compound falls.

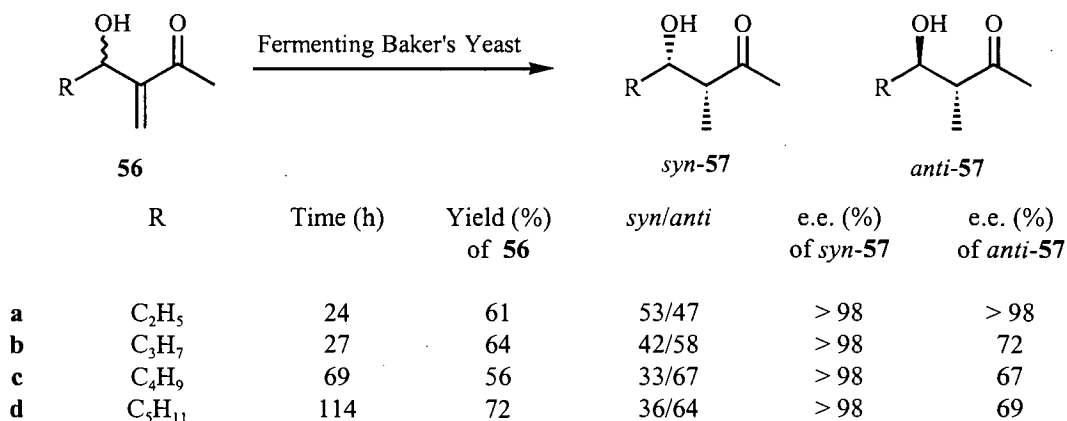


Fig. 1-17 : Baker's Yeast Reduction of β -Hydroxy α -Methylene Ketones..

In recent years the reduction of arylidenecycloalkenes has been studied by Fuganti. Initially it was found that the baker's yeast reduction of **58** produced **59** (30 %, optically pure), **60** (45 %, 10 % e.e.), **61** (trace) and **62** (trace) and that further incubation of **60**, under more forcing conditions, resulted in formation of **61** and **62** in 80 % total yield (Fig. 1-18).⁷⁵ Later studies have shown that the production of

near racemic **60** is due to the keto-enolic equilibrium and that formal hydrogen addition to the double bond of the α,β -unsaturated ketone occurs from the β -*re* face.³⁵

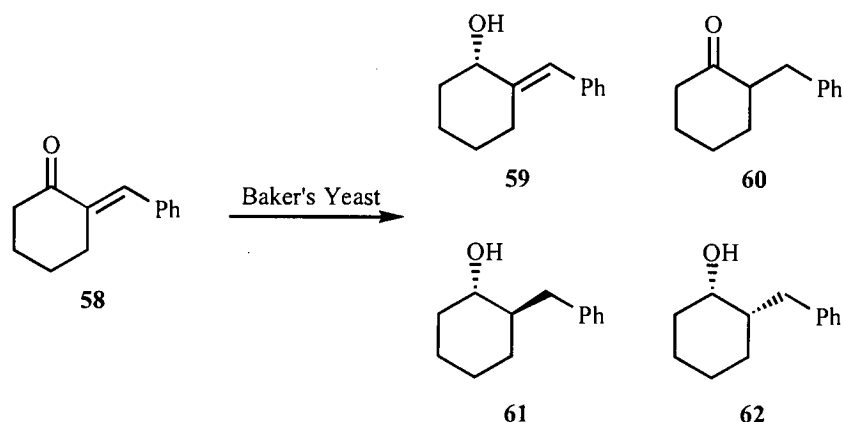
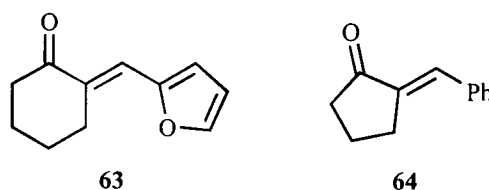


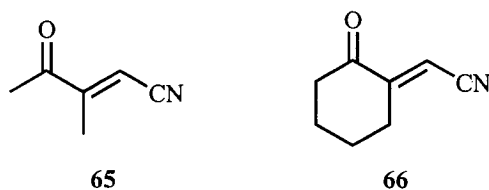
Fig. 1-18 : Baker's Yeast Reduction of Benzylidenecyclohexanone **58**.

Studies on the related compounds **63** and **64** showed a similar distribution of reduction products but the e.e. of the unsaturated alcohol (eg. **59**) decreased on going from **58** to **63** to **64**.³⁵



The ability of baker's yeast to reduce **58** in this manner has been exploited in the synthesis of (*S*)-2-methoxycyclohexanone, a key intermediate in the synthesis of sanfetrinem, and has been shown to be superior in chemo and stereoselectivity to *Nocardia opaca*, *Pichia etchellsii* and *Mucor subtilissimus*.⁷⁶

The reduction of compounds of type **65** and **66** has been studied by Crout *et al.*⁷⁷ Reduction of **65** with baker's yeast, *Zygosaccharomyces rouxii*, *Candida chalmersi*, *Pichia capsulata* and *Pichia farinosa* all afford unsaturated alcohol as the



major product. In the case of **66** baker's yeast produces the unsaturated alcohol as the major product, as does *Candida chalmersi* and *Candida diddensii* whereas *Zygosaccharomyces rouxii* and *Pichia capsulata* produce the unsaturated ketone and *Pichia farinosa* affords the completely saturated compound as the major product. In the case of both **65** and **66** the configuration of the resultant hydroxyl functionalised centre varies between organisms.

1.6.2.3 Reduction of α,β -Unsaturated Aldehydes and Allylic Alcohols.

Baker's yeast reduction of cinnamaldehydes and cinnamyl alcohols to the corresponding dihydroderivatives has been examined in Sect. 1.6.2.1.

The first reported example of the reduction of a non-aromatic allylic alcohol was by Gramatica in 1982. He showed that it was possible to reduce the activated double bond of geranial **67a** and geraniol **67b** to produce (*R*)-(+)-citronellol **68**, whereas neral **69a** and nerol **69b** produced a mixture of *R*- and *S*-**68** in a ratio of 6:4, which is consistent with the partial cis-trans isomerisation of **69a** in the microbiological conversion of nerol into citronellol.⁷⁸ **68** can be oxidised, with selenium dioxide, to **70** which itself can be reduced with baker's yeast to produce **71**, whilst **73** can be produced from **72** (Fig. 1-19).⁷⁹ Three conclusions are drawn from the latter two reactions: no epimerisation occurs at C-6 during the microbial reduction; introduction of hydrogen at C-2 is highly stereospecific and the same absolute stereochemistry is produced at C-2 regardless of that present at C-6.

Baker's yeast has also been shown to reduce the carbonyl activated double bond of **74** and **76** to produce the alcohols **75** and **77**.⁸⁰ Now it can be seen that when the methyl group is β with respect to the carbonyl functionality (eg. **67a**), the hydrogen attacks from the rear (as drawn), but when the methyl group is α to the carbonyl (eg. **72**), it appears that hydrogen is incorporated from the front. This is in agreement with the previously noted *trans* addition of hydrogen in the reduction of cinnamaldehydes

Similarly baker's yeast has been shown to reduce allylic alcohols **78** and **80** (Fig. 1-20).⁸² However, although the same high level of regioselectivity is observed, hydrogen seems to be incorporated from the same side of the molecule at both α and β positions. Although it is possible that this could be as a result of *syn* addition, as has been observed in some vegetal systems,⁸³ it could also be the result of more than one enzyme acting or due to the positioning in the active site.

In addition Santaniello has shown that the terminal methylene group of alcohol **82** can be reduced to produce (2*S*)-3-benzyloxy-2-methyl-1-propanol **83** (Fig. 1-21).⁸⁴

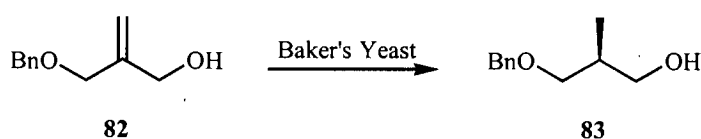


Fig. 1-21 : Baker's Yeast Reduction of an Allylic Alcohol Terminal Double Bond.

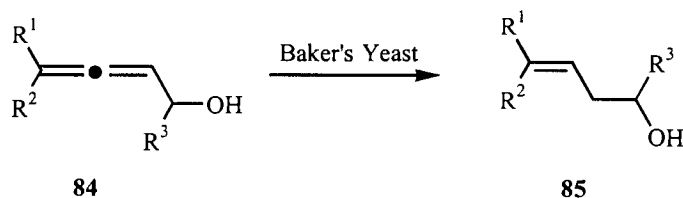
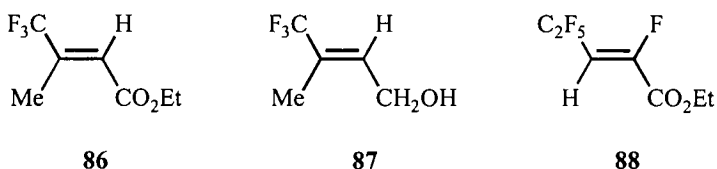


Fig. 1-22 : Baker's Yeast Reduction of α -Allenic Alcohols.

In addition to allylic alcohols primary and secondary α -allenic alcohols **84** can also be reduced by baker's yeast, with the secondary alcohols requiring longer for reduction to occur (Fig. 1-22).⁸⁵

1.6.2.4 Reduction of Halogenated Olefins.

Kitazume showed the capabilities of baker's yeast to reduce the perfluoroalkylated carbon-carbon double bond in **86-88**. All are reduced in good yield (68-72 %), with the esters being recovered intact, and e.e. (67-78 %) although no absolute stereochemistry is discussed.⁸⁶



It has also been shown that reduction of α,β unsaturated compounds where the carbonyl is attached to a perfluoroalkyl group favours production of the saturated ketone (Fig. 1-23), for example reduction of **89** produces both **90** (77 %) and **91** (3 %).⁸⁷ However, when the perfluoroalkyl group is attached to the carbon-carbon double bond reduction favours the saturated alcohol. The reduction of **92** produces **93** (7 %) and **94** (67 %) after 10 d. However, when measured after 2 d the product composition was 81 % **93** and 4 % **94**.

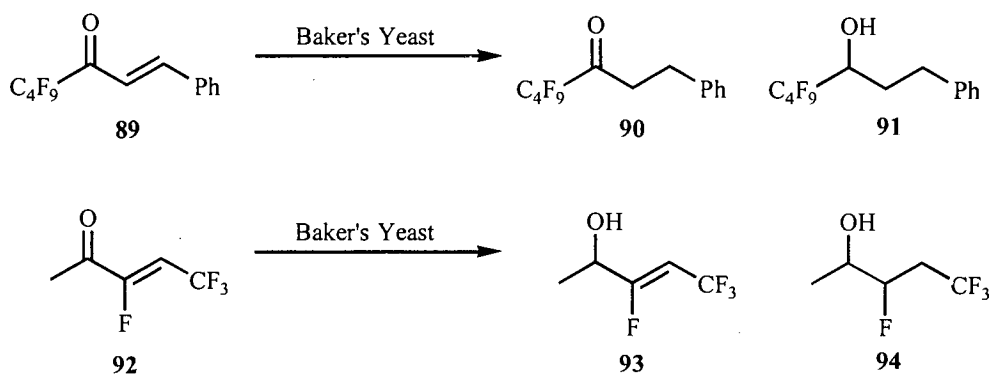


Fig. 1-23 : Reduction of Fluorinated Compounds with Baker's Yeast.

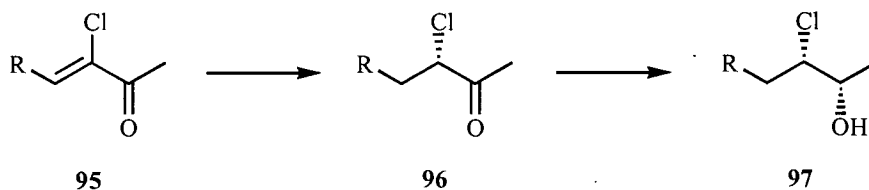


Fig. 1-24 : Reduction of Chlorinated Compounds with Baker's Yeast.

Takeda has studied the reduction of α,β -unsaturated ketones with chlorine incorporated on the double bond **95** ($R = C_2H_5, n-C_5H_{11}, n-C_8H_{17}$).⁸⁸ It was noted that the carbon-carbon double bond is reduced rapidly to produce **96**, and is not affected

by the increasing chain length, whereas the carbonyl reduction is much slower and is impeded by the increasing chain length (Fig. 1-24).

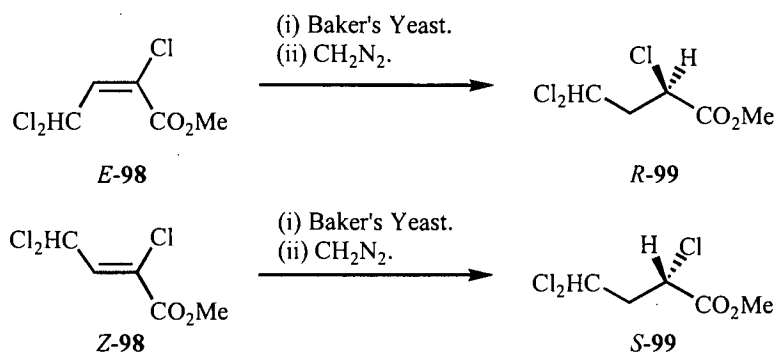


Fig. 1-25 : Baker's Yeast Reduction of *E* and *Z* Isomers to Produce *R* and *S* Enantiomers Respectively.

This example has been developed and utilised in the synthesis of both L- and D-armentomycin.⁸⁹ The key reaction in the synthesis is the baker's yeast reduction of ester **98**, the remarkable feature of which is the formation of *R*-**99** from *E*-**98** and *S*-**99** from *Z*-**98** (Fig. 1-25). The reduced product is isolated as the hydrolysed acid and re-esterification is afforded with diazomethane. *R*-**99** was produced in 65 % (84-92 % e.e.) whilst *S*-**99** was produced in 60 % (97-99 % e.e.) after the two steps.

1.6.2.5 Reduction of α,β -Unsaturated Esters.

Utaka extended the study of halogenated olefins to a larger range of (*E*)- and (*Z*)-methyl 2-chloro-2-alkenoates.⁹⁰ It was found that in each case the reduction products were recovered as the free acids, although ethyl 2,4,4-trichlorobutenate was recovered intact with a trace of the reduced acid, indicating that the ester cannot be the substrate and that hydrolysis must occur before reduction.

2-heptenoic acid and its methyl ester were not reduced; however, the corresponding 2-chloro ester was, indicating that the conjugated carboxyl group in itself is

insufficient to activate the double bond towards reduction. In contrast the 3-chloro-2-alkenoates were not reduced.

This presence of the electron withdrawing chlorine also increases the rate of reaction with **102** being reduced much more quickly than **101** which in turn reacts faster than **100** (Fig. 1-26).

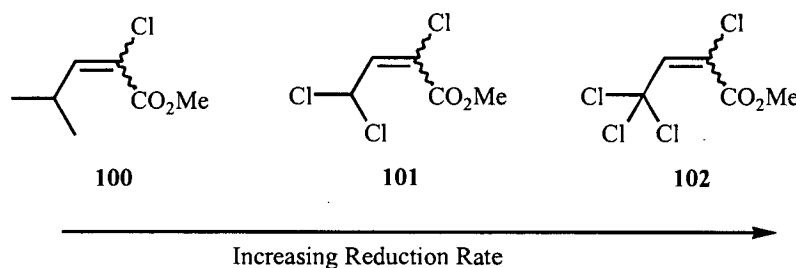


Fig. 1-26 : Relative Reduction Rate in the Baker's Yeast Reduction of 2-Chloro-2-Alkenoates.

The presence of the chlorine also plays an important role in directing the stereochemistry of the product. All of the *Z*-isomers produce *R*-isomers in $\geq 95\%$ e.e., although the *E*-isomers afford products with *S* configuration and the e.e. is generally much lower, 25 - 92 %. It was shown that *E* does not isomerise to *Z* and the lower e.e. values must be due to lower enantioselectivity of the yeast reductase towards the *E*-isomers. The isopropyl group **100** directs the stereochemistry better than ethyl or butyl but not as effectively as the similarly sized highly polar dichloromethyl group **101**.

Leuenberger *et al.* reduced the double bond of ethyl 4,4-dimethoxy-3-methylbut-2-enoate to the corresponding saturated hydroxy ester with baker's yeast as a key step in the synthesis of α -tocopherol.⁹¹

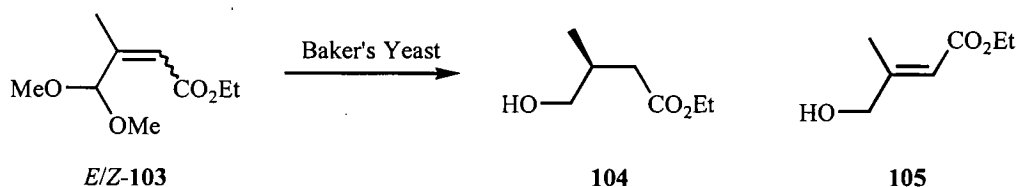
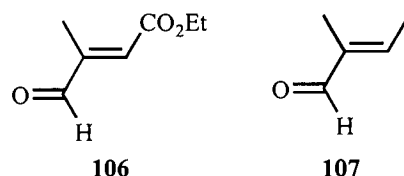


Fig. 1-27 : Baker's Yeast Reduction of an α,β -Unsaturated Ester.

This reduction was examined in greater detail by Ferraboschi who looked at the products from incubation of mixtures of *E*- and *Z*-**103**.⁹² It was noted that the *Z* isomer was reduced to **104** and was the better substrate, whilst the double bond of *E*-**103** was left untouched, although the acetal moiety was hydrolysed and reduced to produce hydroxy ester **105** (Fig. 1-27).

Incubation of hydroxy ester **105** did not produce any reduction product which contrasts with results obtained with the similarly configured aldehydes **70**, **72**, **74** and **76** where the hydroxyl of **105** is replaced by a formyl group and the ester functionality is replaced by an alkyl group.^{79,80}

Incubation of aldehyde **106** does produce some of **104**. This probably occurs *via* formation of the *E*-hydroxy ester which may undergo double bond isomerisation followed by conversion to the aldehyde and then saturation of the double bond to produce **104**. The ratio between



the *E*-hydroxy ester and **104** was 3:1. However, replacement of the ester moiety with the methyl group caused dramatic change in the stereochemical demand of the reaction and the carbon-carbon double bond of **107** was reduced, which is in agreement with the observations of Gramatica *et al.*^{79,80}

104 is a useful chiral synthon and has been incorporated in the synthesis of (25*S*)-26-hydroxycholesterol.⁹³

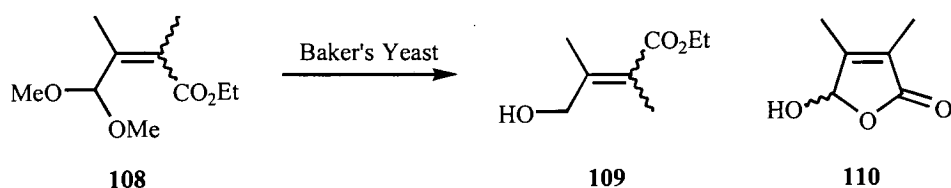
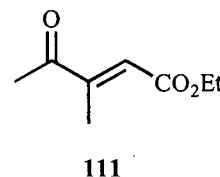


Fig. 1-28 : Baker's Yeast Reduction of a Tetra-Substituted α,β -Unsaturated Ester.

Another interesting result is the attempted reduction of the tetra-substituted double bond of **108**. This was prepared as a probable mixture of *E* and *Z*-isomers and

incubation produced hydroxy ester **109** (probably *E*) and lactone **110**, a fact which highlights the unsuccessful nature of reduction of tetra-substituted double bonds (Fig. 1-28).⁹²

Also reduction of **111** with baker's yeast and *Zygosaccharomyces rouxii* produces the unsaturated hydroxy ester as the major product.⁷⁷ Baker's yeast produces the *S* isomer whilst *Z. rouxii* affords the *R* isomer.



1.6.2.6 Reduction of α,β -Unsaturated Lactones.

The ability of the Basidiomycete *Polyporus durus* to hydrogenate the ring double bond of the α,β -unsaturated lactones 2-decen-5-olide **112** and 2-dodecen-5-olide, from Massoi bark oil, has been reported.⁹⁴ However, Basidiomycetes are slow growing and highly demanding and as such are not particularly useful organisms for biocatalysis. Van der Schaft *et al.* have shown that the same reaction was possible with baker's yeast.⁹⁴

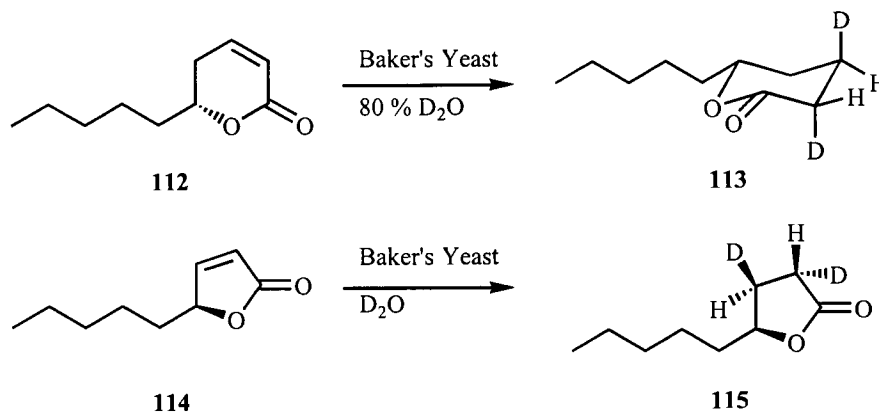


Fig. 1-29 : Determination of the Stereochemical Course of Baker's Yeast Reduction of α,β -Unsaturated Lactones.

Fuganti, Fronza and Grasselli studied this further and determined the stereochemical course by reduction of 2-decen-5-olide **112** with baker's yeast in 80 % D₂O to produce the dideuterated lactone **113** (Fig. 1-29).⁹⁵ It was noted that the hydrogen addition occurs with *trans* stereochemistry and that deuterium was incorporated into

both position 2 and 3 in 70 and 45 % respectively, indicating that the majority of hydrogen added at position 2 arises from water. This indicates the involvement of a reduced pyridine nucleotide coenzyme similar to enoyl-CoA reductases. This is the same *anti* addition that was reported in the cinnamaldehyde reduction.⁴²

Reduction of racemic **112**, where deuterium is incorporated at positions 2 and 3, results in the formation of *R*- and *S*-**113**. In both cases formal *trans* addition of hydrogen from the β -*re* face is observed, irrespective of the stereochemistry at position 5, and with kinetic preference for the *R* isomer.

Studies were extended to the reduction of nonen-4-olide **114**.⁹⁶ Reduction of racemic **114** resulted in a preference for the *S* isomer, although the side-chains are of the same length the e.e. exhibited was substantially lower than that observed for *R*-**113**. It was found that for the γ -lactones the e.e. increased with increasing side-chain length.

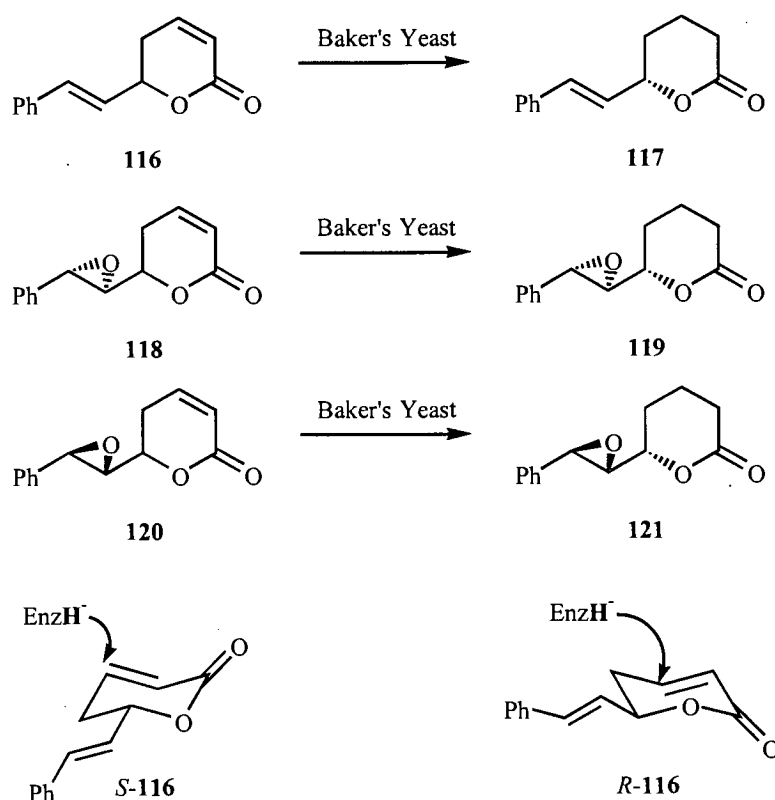


Fig. 1-30 : Baker's Yeast Reduction of Sterically Hindered α,β -Unsaturated Lactones.

However, although the stereochemical preference is inverted, hydrogenation of *S*-114 with baker's yeast in D₂O still occurs by *anti* addition from the β -*re* face to produce *S*-115.

In plants alkan-4-olides usually occur in the *R* configuration. However, in *Dipteryx odorata* nonan-4-olide holds the *S* configuration (20 % e.e.) and accompanies nearly racemic nonen-4-olide. The link between the two is not known, but if one is formed from the other then there is correspondence of the mode of reduction in baker's yeast and in the plant.

In *Cladosporium suaveolens* degradation of racemic ricinoleic acid, and analogues, produces *R*- γ -lactones, whereas isomeric materials, where the hydroxyl group has been moved to odd position, produces *S*- δ -lactones. Higher e.e. was noted in this β -oxidation when chain length was increased and was higher for δ than γ -lactones.⁹⁷

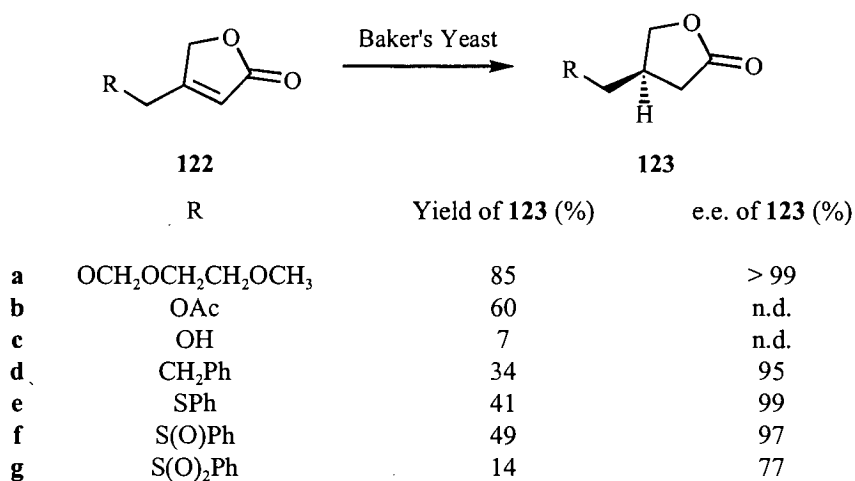


Fig. 1-31 : Baker's Yeast Reduction of Various α,β -Unsaturated Lactones.

It has been reported that during reduction of racemic 116, 118 and 120 the only noticeable reaction is a rapid saturation of the *5S* isomers α,β unsaturated double bonds (Fig. 1-30).⁵⁷ At 50 % conversion the distribution of the four species appears quite similar in all three cases. If the mode of addition is the same as for 112 and 114

then it seems reasonable that the *5S* isomer is easier to reduce (*S*-116 versus *R*-116, Fig. 1-31).

The ability of baker's yeast to reduce α,β unsaturated lactones has been exploited in synthesis. For example Schröer and Welzel have used **123a** in the synthesis of a precursor to (+)-strigol and sorgolactone whilst Takabe has reduced **122b-g** to produce chiral building blocks for terpenoid synthesis and the synthesis of Factor-1.⁹⁸⁻¹⁰⁰

1.6.2.7 Reduction of Sulfur-Substituted Olefins.

Reduction of **124** with baker's yeast produces the saturated alcohol **125** after 3 d which after 19 d is converted to the acid **126** (Fig. 1-32).¹⁰¹ Attempted reduction of (*E*)-3-(1,3-dithian-2-yl)-2-methylpropenoic acid produces no saturated compound indicating that the oxidation occurs after reduction of the double bond. Reduction of the sulfoxide **127** also produces the saturated acid **128**.

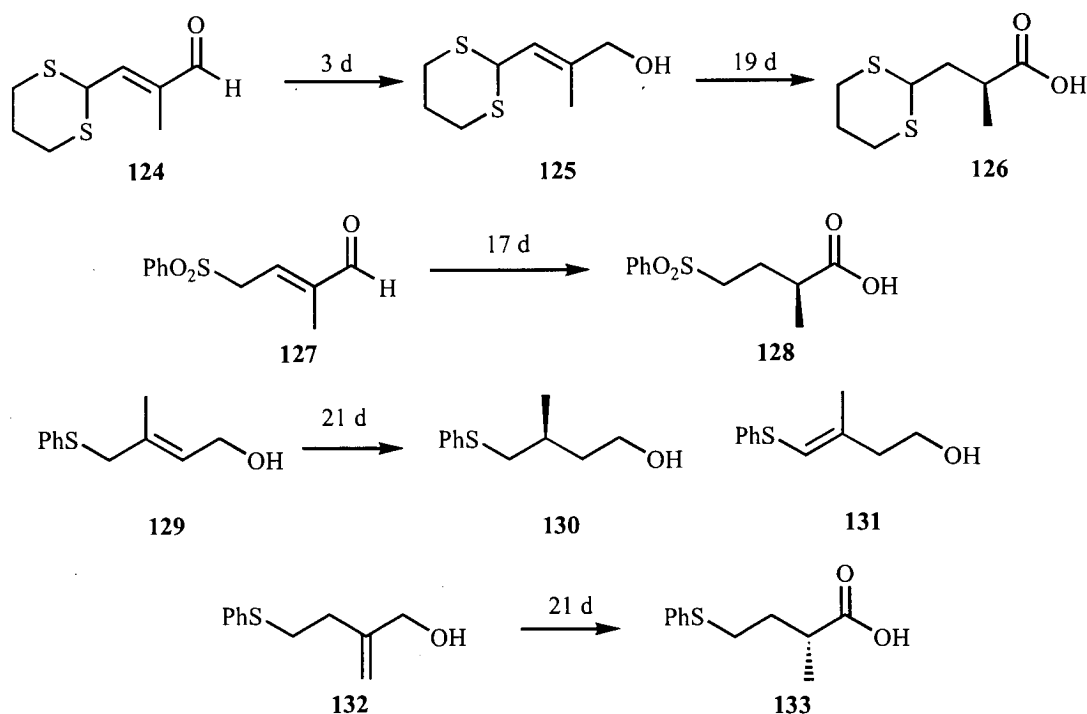
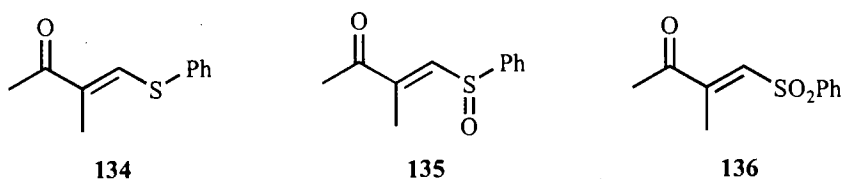


Fig. 1-32: Baker's Yeast Reduction of Sulphated Olefins.

Reduction of the allylic alcohol **129** produces **130** in 22 % and **131**, resulting from double bond migration, in 12 %. However, the incubation of the corresponding sulfone and sulfoxide only produced double bond migration and no reduction, indicating the importance of the nature of the sulfur moiety. Although incubation of the sulfone, corresponding to **129** did not produce any reduced compound **127** did indicating that the position of the methyl group is also important. The vinyl alcohol **132** is also reduced to produce **133**.



Crout *et al* have looked at the reduction of sulfide **134** sulfoxide **135** and sulfone **136** with a variety of organisms.⁷⁷ Baker's yeast reduction of **134** produced both the saturated and unsaturated alcohols in similar amounts; however, reduction with *Zygosaccharomyces rouxii* only produced the unsaturated alcohol with the opposite configuration than that from baker's yeast reduction. In the case of **135** and **136** only carbonyl reduction was observed on incubation with baker's yeast.

1.6.2.8 Reduction of Nitro-olefins.

In 1985 Ohta described the reduction of several β -nitrostyrenes. He had discovered the ability of growing cells of *Rhodococcus rhodocrous* IFO 3338 to reduce the olefinic bond of 1-phenyl-2-nitro propene, 1-(4-hydroxyphenyl)-2-nitropropene and 1-cyclohexyl-2-nitropropene to afford the corresponding propanes.¹⁰² There had been few reports of nitro-olefins previous to this due to their antibiotic activity.¹⁰³

Studies were carried out using 1-phenyl-2-nitropropene and when the substrate was added to growing cells during the logarithmic stage it inhibited growth. The best yields were demonstrated when the substrate was added at the beginning of the stationary phase. Addition of the substrate to a concentration of 0.4 % in acetone (2.5

%) allowed quantitative conversion to product. Addition of a higher concentration of substrate led to lower conversions.

Reduction to the amine followed by derivatisation with (-)-methoxytrifluoromethylphenylacetyl chloride enabled determination of the absolute configuration as *S*. The optically active product was found to racemise, even under slightly acidic conditions. At pH 6.5 the product exhibited 43 % e.e. after 9 h and 27 % e.e. after 48 h.

Later Ohta reported the ability of fermenting baker's yeast to reduce 1-nitro-2-phenylpropene, with the methyl substituent in the α position unlike before (**137**, $R^1 = \text{Ph}$, $R^2 = \text{CH}_3$, Fig. 1-33).¹⁰⁴ The reduced compound was determined to have the *R* configuration and was produced in 50 % yield and 98 % e.e.

The substrate specificity was investigated with the ability of the baker's yeast system to reduce 1-nitro-2-phenylpropenes with chloro, bromo and nitro as *para* substituents on the aromatic ring having been determined.^{104,105}

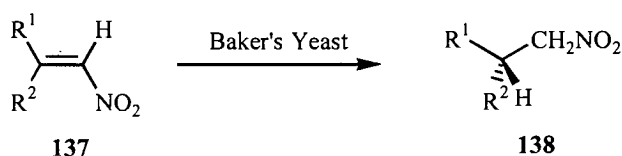


Fig. 1-33: Baker's Yeast Reduction of Nitro Activated Olefins.

It was found that R^2 could be extended to ethyl and propyl groups when R^1 was phenyl, and could be extended to hexyl when R^1 was methyl. All were reduced in reasonable yield to produce the *R* enantiomers with high stereoselectivity.¹⁰⁵

An interesting example was the reduction of *E*- and *Z*-2-methyl-1-nitro-1-octene ($R^1 = \text{hexyl}$, $R^2 = \text{methyl}$ and $R^1 = \text{methyl}$, $R^2 = \text{hexyl}$ respectively) where it was found that both produced the same enantiomer.¹⁰⁵ Two explanations were proposed, firstly that the olefins isomerised during reduction or secondly that the enzyme was

selective for the same enantiotopic face regardless of configuration. Although no definite information was available to distinguish between the explanations, it was noted that the reduction of the exomethylene compound **139** produces the same product **140** as the reduction of 1-nitro-2-phenylpropene **141** (Fig. 1-34). In this case it was expected that the reaction path showed isomerisation of the double bond and indeed control experiments demonstrated a slow equilibrium between **139** and **141**.¹⁰⁵

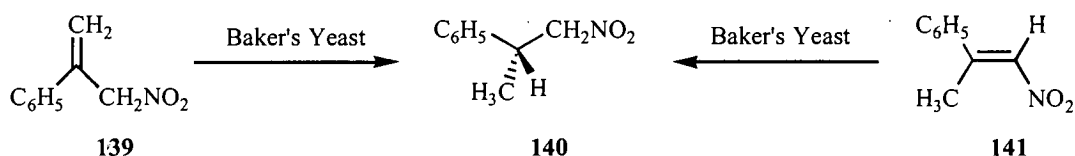
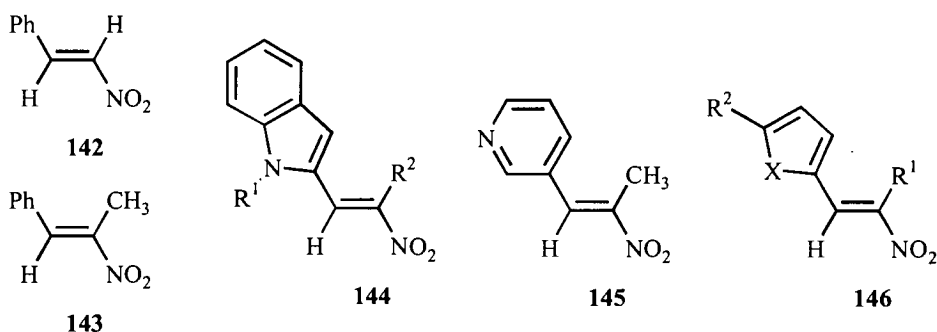


Fig. 1-34: Baker's Yeast Reduction of **139** and **141**

Takeshita further investigated the substrate specificity by showing that both electron withdrawing groups (Cl, Br and CN) and electron donating groups (OCH₃ and CH₃) are acceptable at all three positions on the aromatic ring of **138** and **139**.¹⁰⁶ It was found that the electron donating groups produced higher yields than the electron withdrawing groups.



When a nitro group was incorporated on the phenyl ring of **142** the double bond reduction was accompanied by reduction of the aromatic nitro group when in the *ortho* position, to produce the amine as a minor product. However, similar experiments with **143** found that the amine was produced as the major product in all three cases.

He also extended the process to heteroaromatic nitroalkenes. The indole based **144** was reduced when both R^1 and R^2 were hydrogen and also when R^1 was hydrogen and R^2 was methyl. However it was not reduced when R^1 was phenyl and R^2 was hydrogen. The pyridyl based **145** was also reduced.

Thiophenes, furans and pyrrols were all incorporated into **146**. All were reduced when R^1 and R^2 were hydrogen and when R^1 was hydrogen but R^2 was methyl. The latter compounds were produced in higher yield. Both thiophenes were also reduced when R^1 was bromine.

In 1996 Smallridge reported the reduction of **142** using baker's yeast in light petroleum, with the addition of a small amount of water.¹⁰⁷

The reduction of several *para* substituted analogues of **142** and **143** were reported and it was found that those with electron donating groups produced better yields than those with electron withdrawing groups. This was the same as in the aqueous system, although comparable or higher yields were found in the organic system.¹⁰⁶

The reduction of **143** resulted in the formation of racemic products, as was noted in the aqueous system. In the latter system it was suggested that this was due to the slightly basic reaction conditions.¹⁰² Incubation of 1-phenyl-2-nitropropane under the reaction conditions but with D_2O rather than water showed that racemisation was not occurring in the organic solvent system.

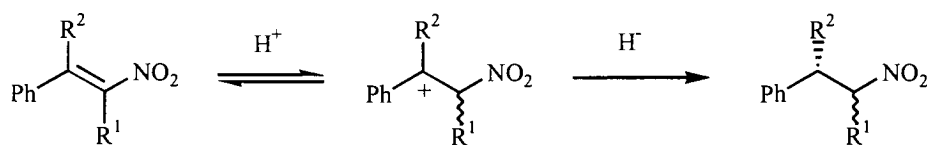


Fig. 1-35 : Mechanism of β -Nitrostyrene Reduction By Baker's Yeast in an Organic Solvent.

It was known that reduction of α -methyl- β -nitrostyrene produced optically active product in the aqueous system and Smallridge later showed, by the reduction of α -deutero- β -nitrostyrene, that this was the case in the organic system.^{61,105}

There followed a study using various deuterated compounds designed to elucidate the mechanism of reduction, and in particular the formation of racemic product (Sect. 5-4). This culminated in the proposal of a mechanism of reduction (Fig. 1-35).⁶¹

1.6.2.9 Miscellaneous.

Although it is generally accepted that only activated double bonds are reduced by baker's yeast an interesting example is the reduction of brachystamide **147** and guineesine **148**.¹⁰⁸

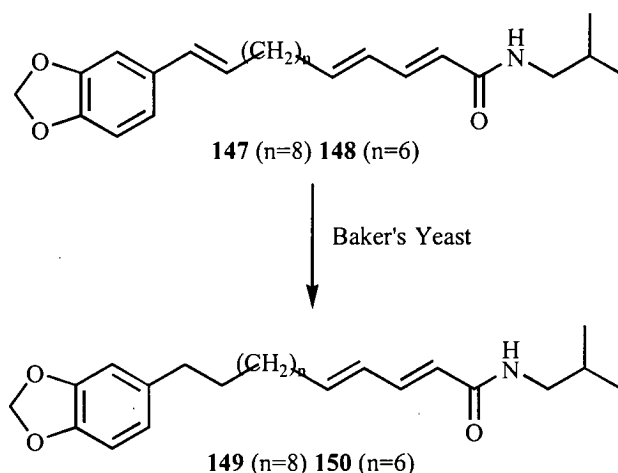
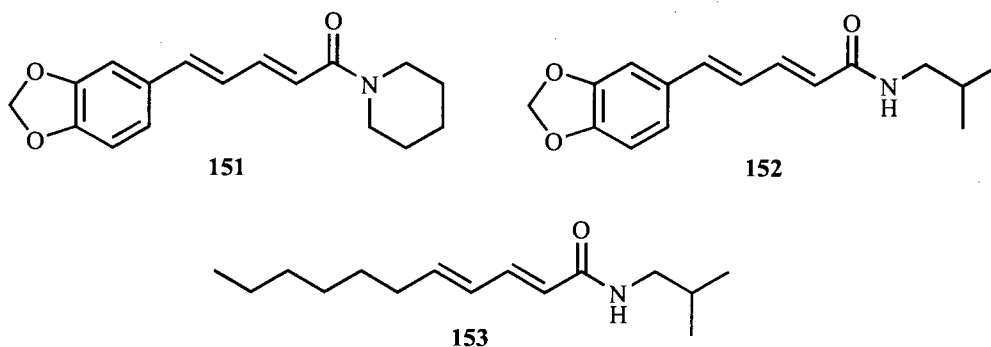


Fig. 1-36 : Baker's Yeast Reduction of an Isolated Styryl Double Bond.

Das has shown that the isolated styryl double bond of enamides **147** and **148** can be reduced in 57 and 62 % respectively with baker's yeast, whilst the rest of the functionality remains untouched (Fig. 1-36). The double bonds of piperine **151**, piperlonguminine **152** and *N*-isobutyl-2*E*,4*E*-decadienamide **153** were left untouched.



1.6.3 Reduction By Other Systems.

Although the majority of whole cell reduction reactions are catalysed using baker's yeast it is important to remember that other organisms are also capable of performing similar reactions. In particular reductions using *Beauveria bassiana* (formerly *Beauveria sulfurescens*) and *Clostridium* spp. have been well studied and isolated incidents exist where other systems, eg. *Nicotiana tabacum*, have been utilised.

1.6.3.1 Beauveria bassiana.

Kergomard and co-workers have shown the ability of this organism to reduce a variety of α,β -unsaturated double bonds, with the reduction of α,β -unsaturated ketones having been extended to a variety of fungi.¹⁰⁹

This was discovered serendipitously during a study with 2-methylcyclopentenone.¹¹⁰ It was envisaged that the organism would hydroxylate the acycle but the only isolated product was (*R*)-2-methylcyclopentanone in 90 % yield. However, it was noted that to achieve such reduction the reaction must be carried out with minimum aeration and that reduction did not occur with 3-methyl cyclopenten-2-one. Also reduction of racemic 2,5-dimethyl-2-cyclopenten-2-one shows a kinetic preference for the 5*R* isomer.¹¹¹

Studies were extended to cyclohex-2-enones which produced both saturated ketones and alcohols. It has been shown that the double bond of cyclohex-2-enones, substituted with methyl groups in the 4-, 5- or 6- positions are reduced as are (+)- and (-)-carvone. However, 2,6-dimethylcyclohex-2-enone and 2,6,6-trimethylcyclohex-2-enone were not reduced.¹¹²

A variety of acyclic aldehydes and ketones were also reduced by *Beauveria bassiana*.¹¹²⁻¹¹⁴ Studies of the reduction of α,β -unsaturated aldehydes showed that double bond saturation precedes aldehyde reduction. In the case of both acyclic and cyclic double bonds it was noted that at least one hydrogen has to be present at the β position on the double bond.

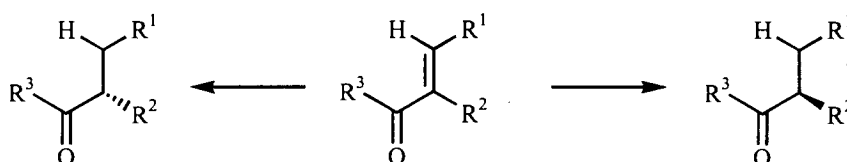


Fig. 1-37: *Beauveria bassiana* Saturation of α,β -unsaturated Enones.

The mechanism of hydrogenation and the stereochemistry of this fungal reduction have also been studied. It was noted that attack of hydrogen at the α carbon occurs from the rear if the larger substituent is on the left and from the front if the larger substituent is on the right (Fig. 1-37).^{112,114} It was also found to incorporate a *trans* addition of hydrogen across the double-bond, being incorporated from the α -*si* and β -*re* faces.^{110,115-117}

1.6.3.2 *Clostridium* spp..

The majority of the work concerning these anaerobic bacteria has been carried out by Simon and co-workers. Although they have shown that it is possible to hydrogenate a variety of substrates, including allylic alcohols and α,β -unsaturated aldehydes,¹¹⁸⁻¹²⁰

the majority of their work has concentrated upon the reduction of 2-enoates.^{41,121-123} It was noted that the carbon-carbon double bond of these 2-enoates does not require an activating group other than the carboxylic acid moiety.

These reactions are performed under an atmosphere of hydrogen. Anaerobic organisms contain hydrogenase enzymes capable of oxidising dihydrogen whilst reducing a natural electron mediator such as ferredoxin. This mediator can then regenerate NADH from NAD for use in the reduction reaction.⁴¹

It has also been shown that this natural electron mediator can be replaced by an artificial one, such as methyl viologen. The use of an artificial mediator can replace one of the three enzymes because the enoate reductase can use the artificial mediator directly, without the need for ferredoxin and ferredoxin-NAD reductase, and the hydrogenase enzyme can reduce the viologen.⁴¹ It is also possible to regenerate the methyl viologen electrochemically so that only the enoate reductase is required.^{41,124}

It has been determined that saturation of the double bond occurs with *trans* incorporation of hydrogen and reactions have been carried out using resting cells and purified enoate reductases.^{41,125-127} This has been used in the reduction of the monomethyl fumarates **154** and **156** to produce compounds that are chiral by nature of the incorporation of hydrogen isotopes (Fig. 1-38).¹²⁸

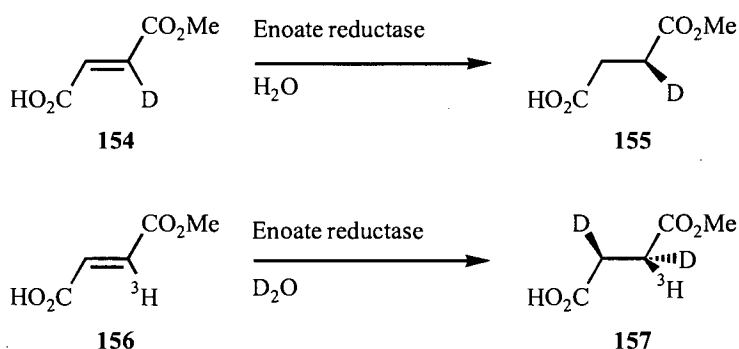


Fig. 1-38 : Reduction of Monomethyl Fumarates **154** and **156** by an Enoate Reductase from *Clostridium* spp..

Although whole cells of various *Clostridium* spp. were capable of reducing allylic alcohols the purified enoate reductase was not and this reaction required the introduction of an aldehyde dehydrogenase. It was noted that the α,β -unsaturated alcohol was firstly dehydrogenated to form the 2-enal which was reduced to the saturated aldehyde before carbonyl reduction to afford the saturated alcohol.¹¹⁹

Kergomard found that the reduction of α,β -unsaturated acids could not be achieved using *Beauveria bassiana* and turned his attention to growing *Clostridium* spp., in the anticipation that this would provide a simplified procedure. This system was used to reduce the carbon-carbon double bond of both 2-deuterio and 3-deuterio cyclohex-2-enone which provided further evidence for a *trans* incorporation of hydrogen. However, yields with growing cells were generally less than with isolated ones.^{129,130}

This reduction of 2-deuterio-2-cyclohexenone was also found to be in agreement with the rule proposed by Kergomard to determine the direction of attack α to the carbonyl in *Beauveria bassiana* mediated reductions (Fig. 1-37).

1.6.3.3 *Nicotiana tabacum*.

The cultured cells of *Nicotiana tabacum* are unusual in that reduction occurs in a *syn* fashion.^{83,131} This has been shown to occur in the reduction of (1*S*,5*S*)-verbenone **158** which is reduced to produce (1*S*,2*R*,5*S*)-*cis*-verbanone **159** (Fig. 1-39). None of the *trans* isomer is produced and (1*R*,5*S*)-**158** is only reduced in trace amounts, indicating that the reduction is both enantio and stereospecific.

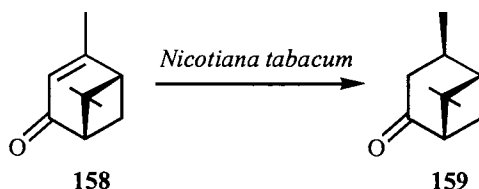


Fig. 1-39 : *Nicotiana tabacum* Mediated Reduction of (1*S*,5*S*)-Verbenone **158**.

It has also been shown that saturation of the double bond occurs *via re-re* attack, with the hydrogen incorporated at C-2 arising from the *pro-4S* position of NADPH whilst that at C-3 arises from the medium.

1.6.3.4 *Streptomyces cinereocrocat*.

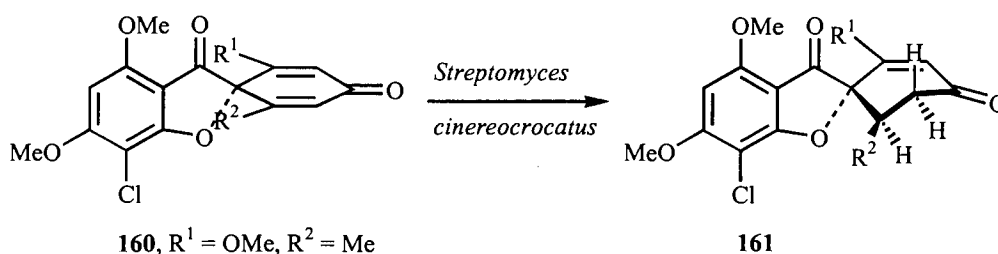
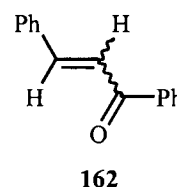


Fig. 1-40 *Streptomyces cinereocrocat* Mediated Reduction of (-)-Dihydrogriseofulvin 160

Sato has studied the *Streptomyces cinereocrocat* mediated reduction of (-)-dihydrogriseofulvin 160 to produce (+)-griseofulvin 161.¹³² It has been shown that this occurs by *anti* addition with the hydrogen incorporated α to the carbonyl arising from the *pro-4R* position of NADPH whilst that β to the carbonyl is delivered from the reaction medium.

1.6.3.5 *Corynebacterium equi* IFO 3730.

Ohta has demonstrated the ability of *Corynebacterium equi* IFO 3730 to reduce the double bond of chalcone 162; however, the regiochemistry of 162 is not discussed.^{133,134}



Reduction was possible with a variety of both electron donating and electron withdrawing groups in the *para* position of the phenyl rings and yields could be excellent with the unsubstituted chalcone exhibiting quantitative conversion and

isolated yield. No carbonyl reduction was detected and, when present, aromatic nitro groups were left untouched.

1.6.4 Metabolic Pathways.

The enzymes being utilised during a biotransformation have a natural role in the life cycle and metabolism of the cell. Carbon-carbon double bond reduction plays an important role in these metabolic pathways, for example in the synthesis of fatty acids and steroids.

Enoyl reductase enzymes catalyse the final step in the condensation-reduction-dehydration-reduction cycle that lengthens the chain of a fatty acid by $-\text{CH}_2\text{CH}_2-$ at each turn of the cycle. In 1980 Sedgewick reported the mechanism of double bond reduction in yeast.¹³⁵ This was found to involve *anti* addition with 2-*si*, 3-*si* attack on the double bond with the hydrogen at C-3 being delivered from the *pro*-4*R* position of NADPH and that at C-2 being delivered by water (Fig. 1-41).

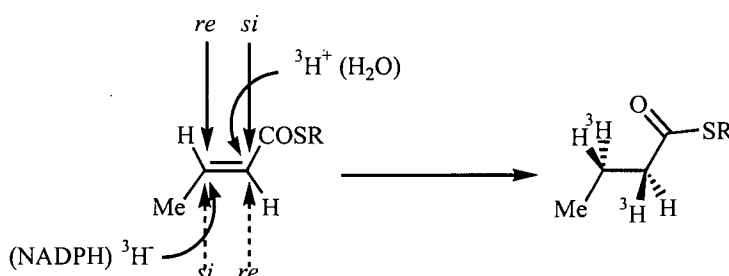


Fig. 1-41 : Stereochemical Course of Yeast Enoyl Reductase Carbon-Carbon Double Bond Saturation.

It has been shown that the 5 α -reductase from *Penicillium decumbens* reduces the double bond of the deuterated testosterone **163** to the 5 α -dihydrotestosterone **164** in an *anti* fashion.¹³⁶

Biosynthetic studies with *Streptomyces collinus* have shown that the cyclohexanecarboxylic acid moiety of anastrienin A **167** is derived from shikimic acid **165** via 1-cyclohexenecarboxylic acid **166** (Fig. 1-43).¹³⁷

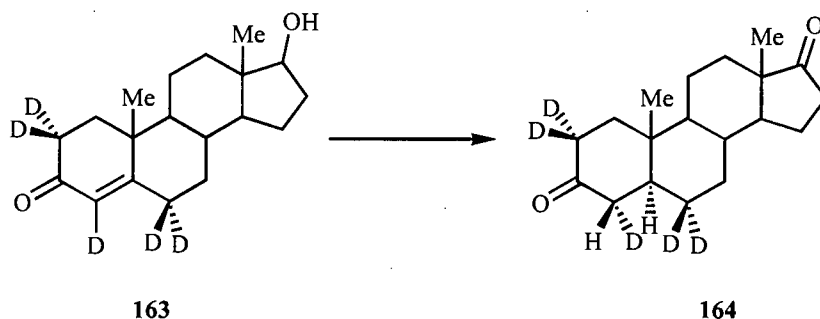


Fig. 1-42 : Stereochemical Course of Reduction of a Steroidal Double Bond by the 5 α -Reductase from *Penicillium decumbens*.

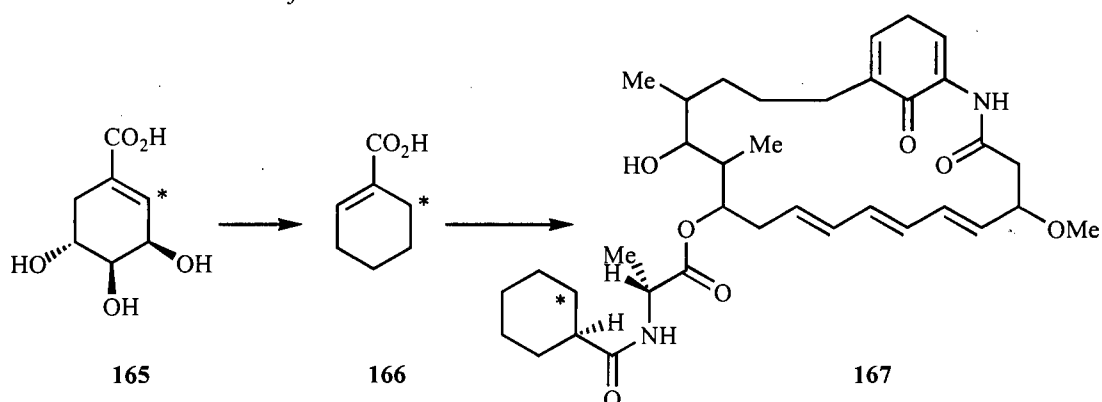


Fig. 1-43 : Stereochemical Fate of D-(-)-[2-¹³C]Shikimic Acid in the Conversion to the Cyclohexylcarbonyl Moiety of Anastrienin A **167**.

It was noted that a NADPH-specific enoyl-CoA reductase from *Streptomyces collinus* converts **168** to **169** by hydride delivery from the *pro*-4S position of NADPH to the *si* face of the β -carbon of **168** and *anti* incorporation of hydrogen from solvent at the α -carbon (Fig. 1-44).

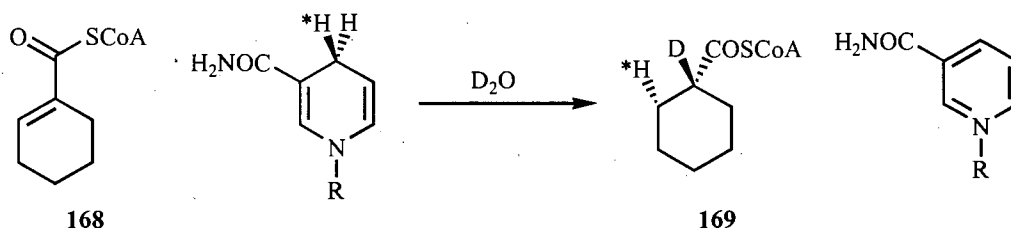


Fig. 1-44 : Reduction of **168** With an NADPH-Specific Enoyl-CoA Reductase From *Streptomyces collinus*.

2. Results & Discussion : Synthesis of Potential Substrates.

This chapter deals with the synthesis of a range of potential substrates for the *Rhodotorula rubra* CBS 6469 biotransformation.

The mainstay of this synthesis is the Knoevenagel condensation between a range of benzaldehydes and benzophenones with various five membered heterocycles to produce compounds based on (*Z*)-5-(benzylidene)thiazolidine-2,4-dione, with modified substitution patterns and varying constituent atoms of the heterocyclic ring (Fig. 2-1).

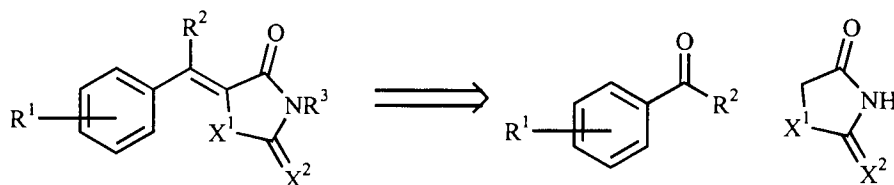


Fig. 2-1 : Retrosynthesis of Potential Substrates Based Upon (*Z*)-5-(benzylidene)thiazolidine-2,4-dione.

This condensation reaction will be discussed initially, along with the associated regiochemistry of the products, followed by the synthesis of the non-commercially available aldehydes. The next sections focus on the synthesis of the benzylidene precursors to the Pfizer drug Englitazone and a Lilly compound which has also shown pharmacological activity towards NIDDM. The final section discusses expansion of the template including the synthesis of one alkene where the phenyl ring has been replaced by a cyclohexyl ring. This also includes the ring opening of (*Z*)-5-(4-methoxybenzylidene)-2-thione-thiazolidine-4-one and subsequent functional group transformations to produce a class of compounds based upon (*Z*)-2-sulfanyl-3-phenylpropenoic acid.

2.1 Synthesis of Potential Substrates Based Upon 5-Benzylidene Thiazolidine-2,4-dione.

2.1.1 Knoevenagel Condensation of Various Benzaldehydes and Benzophenones With Five Membered Heterocycles.

The Knoevenagel condensation belongs to the family of reactions, based around the Aldol reaction, involving the attack of a carbon-hetero atom multiple bond by a carbanion. The Knoevenagel reaction involves the attack of an aldehyde or ketone, usually without α hydrogens, by the carbanion of a carbon centre flanked by two electron withdrawing groups to stabilise the negative charge.¹³⁸

By changing the heterocycle involved it was possible to vary X^1 and X^2 . It was also possible to change R^1 by changing the substituent and substitution pattern on the carbonyl compound used, whilst using a ketone instead of an aldehyde introduced substitution at R^2 (Fig. 2-2 and Table 2-1). After the condensation step it was possible to protect the ring nitrogen to introduce substitution at R^3 .

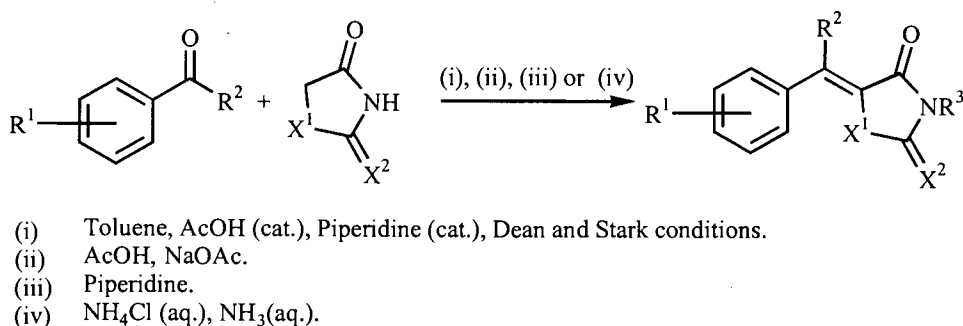


Fig. 2-2 : Knoevenagel Condensation.

Although four sets of reaction conditions were employed the majority of the compounds, **170-191**, resulted from the condensation of 4-substituted benzaldehydes, or benzaldehyde itself, with thiazolidine-2,4-dione. All of these, except **189** and **190**, were prepared by heating at reflux in toluene, under Dean and Stark conditions, with

acetic acid and piperidine as catalyst. All were prepared in moderate to good yield (62-90 %) except for **176**, **180** and **186-188** which exhibited yields between 34-44 %.

None of the yields shown were optimised and all aldehyde condensations were complete within the reaction time shown. **171-188** were purified by recrystallisation from ethanol and it is possible that the yields could have been improved by use of a different solvent for this procedure. However, it was imperative that these compounds could be produced quickly and ethanol appeared to be a universal solvent for their purification.

189 and **190** were prepared, in 77 and 78 % yield respectively, by heating the appropriate benzaldehyde and thiazolidine-2,4-dione at reflux in acetic acid with sodium acetate for 3 and 21 h respectively. It has been reported that these compounds were not formed under the Dean and Stark procedure; however, attempts to produce **189** by this method resulted in 96 % crude yield.¹³⁹

Similarly, thiazolidine-2,4-dione was condensed with 2-, 3- and 2,6-dimethoxy benzaldehydes to produce **197-199** under the Dean and Stark conditions in 77-82 % yield, after recrystallisation from ethanol. **200** resulted from a similar condensation with 3,5-di-*tert*-butyl-4-hydroxy benzaldehyde in 80 % yield on recrystallisation from toluene.

Condensations were carried out between thiazolidine-2,4-dione and three benzophenones to produce potential substrates with tetra-substituted double bonds, **201**, **202** and **203** in 52, 29 and 50 % yield respectively. The reaction times increased relative to the corresponding benzaldehydes and as the length of the alkyl chain increased. The condensations were allowed to proceed for 50 h, 47 h and 10 d respectively; however, none had produced quantitative conversion to product within this time. **201** and **202** were purified by recrystallisation from toluene, with the latter requiring further purification by column chromatography, while **203** was purified by column chromatography alone.

	R ¹	R ²	R ³	X ¹	X ²	Conditions	Reaction Time (h)	Yield (%)	Z/E	δ_{H} (ppm)	Lit. δ_{H} (ppm) ^a	³ J _{C,H} (Hz)
170	H	H	H	S	O	(i)	27	80	Z	7.77	7.85	6.5
171	4-OH	H	H	S	O	(i)	27	76	Z	7.69		6.5
172	4-OMe	H	H	S	O	(i)	25.5	75	Z	7.72		6.5
173	4-OEt	H	H	S	O	(i)	20	65	Z	7.68		6.5
174	4-OPr	H	H	S	O	(i)	18	71	Z	7.68		
175	4-OBu	H	H	S	O	(i)	22.5	84	Z	7.71		
176	4-OPr ⁱ	H	H	S	O	(i)	18	44	Z	7.74		6.5
177	4-OBu ⁱ	H	H	S	O	(i)	18	64	Z	7.69		
178	4-OBn	H	H	S	O	(i)	24	82	Z	7.74		6.5
179	4-SMe	H	H	S	O	(i)	24	72	Z	7.69		6.5
180	4-Me	H	H	S	O	(i)	24	37	Z	7.74		
181	4-Et	H	H	S	O	(i)	24	78	Z	7.70		6.5
182	4-Pr	H	H	S	O	(i)	22	90	Z	7.71		
183	4-Bu	H	H	S	O	(i)	22	88	Z	7.69		
184	4-Bn	H	H	S	O	(i)	22	88	Z	7.74		6.5
185	4-F	H	H	S	O	(i)	24	65	Z	7.80		6.5
186	4-Cl	H	H	S	O	(i)	47	40	Z	7.74		
187	4-Br	H	H	S	O	(i)	24	41	Z	7.76		
188	4-I	H	H	S	O	(i)	30	34	Z	7.87		6.5

^a References to literature data are included in chapter 6 with the details of the synthesis of each of these compounds.

Table 2-1 : Result of Knoevenagel Condensations - Conditions of Reaction, Reaction Time, Yield and Resulting Regio-chemistry.

	R ¹	R ²	R ³	X ¹	X ²	Conditions	Reaction Time	Yield (%)	Z/E	δ_H (ppm)	Lit. δ_H (ppm) ^a	³ J _{C,H}
189	4-CN	H	H	S	O	(ii)	21 h	77	Z	7.59		6.0
190	4-NO ₂	H	H	S	O	(ii)	3 h	78	Z	7.81		6.5
191	4-OCF ₃	H	H	S	O	(i)	30 h	62	Z	7.77		6.5
192	4-OMe	H	H	NH	O	(iii)	75 min	37	Z	6.39	6.40	
193	4-Cl	H	H	NH	O	(iii)	100 min	26	Z	6.40	6.40	
194	4-CN	H	H	NH	O	(iii)	30 min	26	Z	6.44	6.44	
195	4-OMe	H	H	S	NH	(i)	21 h	30	Z	7.55		5.5
196	4-OMe	H	H	S	S	(iv)	5 min	59	Z	7.56		6.0
197	2-OMe	H	H	S	O	(i)	24 h	77	Z	7.94		7.0
198	3-OMe	H	H	S	O	(i)	24 h	81	Z	7.75		6.5
199	2,6-OMe	H	H	S	O	(i)	18 h	82	Z	7.90		7.0
200	3,5-Bu ^t -4-OH	H	H	S	O	(i)	36 h	80	Z	7.73	7.77	6.5
201	H	Me	H	S	O	(i)	50 h	52	Z			
202	4-OMe	Me	H	S	O	(i)	47 h	29	Z			
203	4-OMe	Et	H	S	O	(i)	10 d	50	Z			

^a References to literature data are included in chapter 6 with the details of the synthesis of each of these compounds.

Table 2-1 : Result of Knoevenagel Condensations - Conditions of Reaction, Reaction Time, Yield and Resulting Regio-chemistry.

By carrying out the condensation using imidazolidine-2,4-dione (hydantoin) instead of thiazolidine-2,4-dione it was possible to switch the ring sulfur in the benzylidene compound for nitrogen. Three such compounds were made, **192-194**, utilising the procedure reported by Billek, by heating to 130 °C in piperidine at a concentration of 5 moldm⁻³ for between 30 and 100 min.¹⁴⁰ Due to the nature of the reaction conditions, monitoring by tlc proved extremely difficult. Each of the three compounds were produced in poor yield on recrystallisation from acetic acid and washing with ethanol (37, 26 and 26 % respectively).

196 and **195** have the carbonyl at position 2 replaced by a thiocarbonyl and imine respectively. The latter was prepared by the Dean and Stark procedure, using 4-methoxybenzaldehyde and thiazolidine-2-imine-4-one (pseudothiohydantoin), in 30 % yield on recrystallisation from acetic acid, followed by washing with water and diethyl ether. The low yield arises due to polarity of the pseudothiohydantoin and **195** which makes monitoring the procedure by tlc difficult as well as the purification of the compound. The former was synthesised, by Gaudry and McIvor's procedure, in 59 %, by condensing 4-methoxy benzaldehyde with thiazolidine-2-thione-4-one (rhodanine) in aqueous ammonia and ammonium chloride followed by recrystallisation from ethanol.¹⁴¹ However, it was possible to produce **196** using the Dean and Stark method in 55 % on recrystallisation from ethanol.

2.1.2 Protection of Ring Nitrogen.

The final position that has been changed is R³. This was achieved by treating **172** with sodium hydride in THF and DMF followed by methyl iodide or benzyl bromide to produce **204** and **205** respectively. Both were produced in quantitative yield (Fig. 2-3).

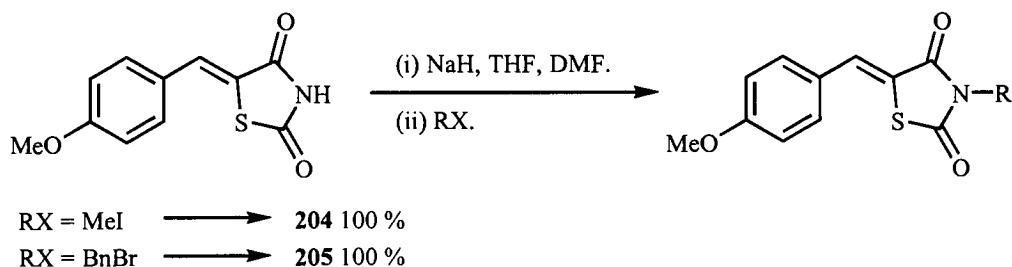


Fig. 2-3 : Protection of Ring Nitrogen.

2.1.3 Regio-chemistry of Benzyldiene Compounds.

In the case of the three hydantoin based compounds **192-194** a comparison can be made between δ_{H} of the olefinic proton in these three compounds and that reported in the literature for the *Z* isomers. From Table 2-1 it can be seen that in all three cases these values are in good agreement.

However, there is a lack of reports concerning characterisation data for pure isomers of the majority of the potential substrates which makes the assignment of *E/Z* more difficult.

Vogeli and von Philipsborn have shown that the regiochemistry about the double bond of tri-substituted alkenes can be determined by observing the $^3J_{\text{C,H}}$ vicinal coupling in the ^1H coupled ^{13}C nmr spectra.¹⁴² Although these values have been used to determine the double bond geometry in several natural products it has been noted that unless good model compounds are available this alone is not an adequate means of determining the regiochemistry as the $J_{\text{trans}}/J_{\text{cis}}$ ratio can vary.

It has been shown that these differences are due to a number of factors including substituent electronegativity, π bond order, bond angle, torsional angle, steric interactions and the hybridisation of the ^{13}C involved in the coupling.

The closest models available are a range of benzylidene 5(4*H*)-oxazolones where these constants have been measured to be 12.5 and 5.5 Hz for the *trans* and *cis* coupling respectively.¹⁴³ The former corresponds to *E* geometry about the double bond and the latter to the *Z* geometry (Fig. 2-4). Although the heterocyclic ring of the potential substrates differs from the 5(4*H*)-oxazolones the variations in π bond order, bond angle, torsional angle and steric interactions were expected to be negligible, and hence have little effect on the $^3J_{C,H}$ between classes of compounds. The main difference was the change in electronegativity of a substituent attached directly to the double bond as the nitrogen had been replaced by sulfur in most cases.

Vogeli and von Philipsborn examined each of the contributing aspects individually, on a range of simple ethylene based compounds, and found that an increase in electronegativity of an atom attached directly to the double bond resulted in a slight decrease of both *cis* and *trans* $^3J_{C,H}$. Replacing the nitrogen with sulfur would increase the $^3J_{C,H}$ values as the electronegativity has decreased.

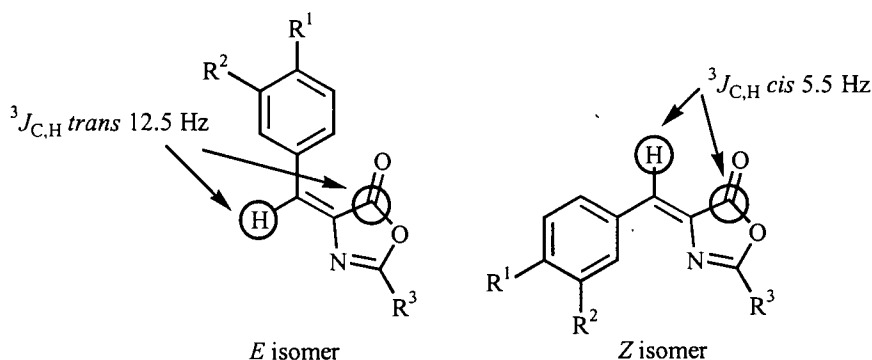


Fig. 2-4 : $^3J_{C,H}$ Coupling Used To Determine Regio-chemistry About the Double Bond In 4-Benzylidene-2-substituted-5(4*H*)-oxazolones.

X-ray crystallography has shown that **172**, **179** and **181** are all *Z* isomers (Appendix 2). The latter two produced yellow needles and colourless plates on crystallisation from ethanol while the former was crystallised as its DMSO solvate. This was achieved by diffusion of diethyl ether into a saturated solution of **172** in DMSO to produce a colourless block. The vicinal $^3J_{C,H}$ of these three compounds has been

measured, at 63 MHz in DMSO- d_6 , and found to be 6.5 Hz, which appears to be in good accordance with the anticipated value of $^3J_{C,H}$ for a *cis* interaction arising from *Z* geometry about the double bond.

Table 2-1 shows that, with the exception of **189** where $^3J_{C,H} = 6.0$ Hz, the majority of the thiazolidine-2,4-dione based compounds exhibited $^3J_{C,H} = 6.5$ Hz. Although this coupling has not been measured for all such compounds there is enough evidence to assume the formation of the *Z* isomer in each case.

196 and **195** possessed $^3J_{C,H}$ values of 6.0 and 5.5 Hz respectively. Although this differs slightly from the thiazolidine-2,4-dione based compounds it is still indicative of the *Z* configuration, with the difference presumably arising from the differing electronegativity of the functionality at position 4 of the heterocycle.

Although this system is useful when dealing with a tri-substituted double bond the incorporation of an alkyl group at R^2 makes the determination of the double bond geometry more difficult. Due to the difference in shielding of the olefinic proton between regioisomers there is a distinct difference in δ_H of the olefinic proton in tri-substituted compounds. It would be expected that a similar scenario would exist for the tetra-substituted compounds **201-203**. In each of these compounds there was only one signal for the alkyl group at R^2 . As it is clear that the phenyl ring prefers a *trans* relationship to the carbonyl compound, it can be assumed that the *Z* isomers are formed as it is unlikely a small alkyl group imparts a large enough effect to completely reverse the regiochemistry of these compounds.

In the case of **204** and **205** the procedure for protection of the ring nitrogen should not have caused isomerisation of the double bond and it is assumed that these retain the *Z* geometry inherent from **172**.

2.2 Synthesis of Non-Commercially Available Aldehydes.

2.2.1 Synthesis of 4-Iodobenzaldehyde **209** and (Z)-5-(4-methoxybenzylidene-*d*)thiazolidine-2,4-dione **215**.

Although it is possible to carry out the transformation from acid to aldehyde more directly the use of well established reactions has certain advantages. As with the Knoevenagel condensations there was emphasis on a quick, versatile route to various compounds and the chosen route provides this. There was little development involved and the route is adaptable to the synthesis of other benzaldehydes from any of the appropriate commercially available intermediates.

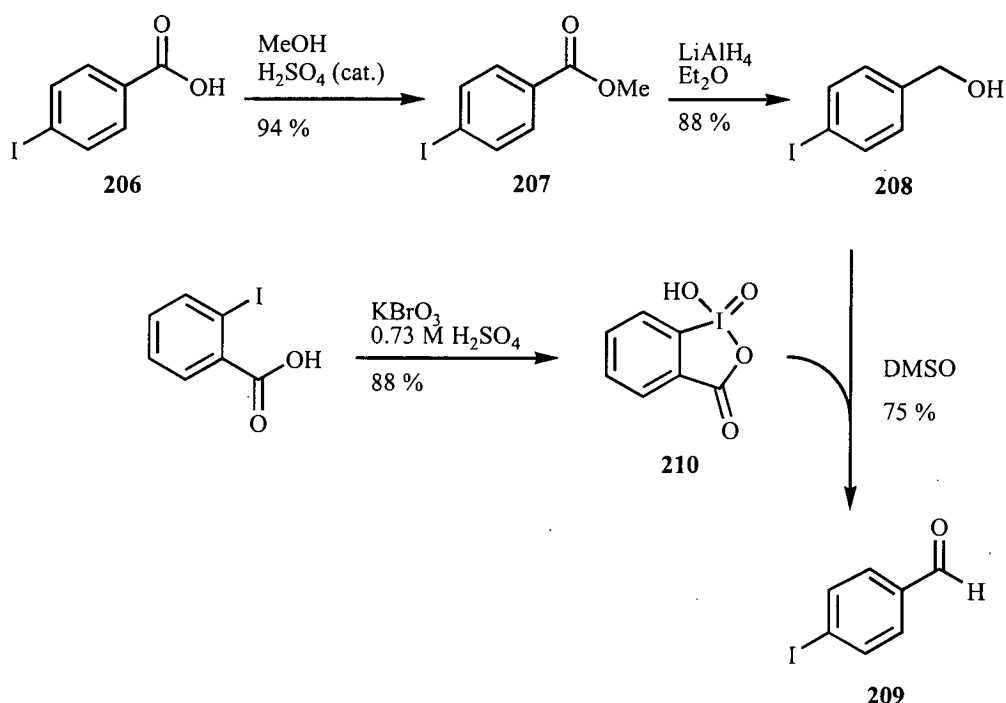


Fig. 2-5 : Synthetic Route to 4-Iodobenzaldehyde **209**.

All steps are easily carried out with simple purification of the crystalline intermediates. The esterification of 4-iodobenzoic acid **206** was complete after heating at reflux overnight producing ester **207**, in 94 % yield after recrystallisation from methanol. Subsequent reduction of ester **207** by lithium aluminium hydride for

1 h produced alcohol **208** in 88 % after purification by column chromatography.¹⁴⁴ This was oxidised to aldehyde **209**, in 75 % after column chromatography, by treatment with IBX **210** in DMSO for 15 min. IBX **210** itself was readily prepared by treatment of 2-iodobenzoic acid in aqueous sulfuric acid with potassium bromate.¹⁴⁵

It was possible to use crude **207** and **208** in the next stage, although **209** required column chromatography to remove traces of DMSO. This meant that without further purification of the intermediate compounds acid **206** can be transformed into aldehyde **209** within twenty four hours. The aldehyde **209** was then condensed with thiazolidine-2,4-dione (Sect. 2.1.1).

This route also allowed the introduction of deuterium by reduction of the ester with lithium aluminium deuteride. This has been utilised in the synthesis of 4-methoxybenzaldehyde-*d* **214** (Fig. 2-6).

The ester **212** was prepared in almost quantitative yield from the acid **211**, without further purification, which in turn was reduced with lithium aluminium deuteride to produce the *di*-deutero 4-methoxy benzyl alcohol **213** in 68 %, after column chromatography. Again esterification was complete after heating at reflux overnight and the reduction took four hours. Alcohol **213** was oxidised smoothly with IBX **210** in 45 min to produce the deuterated aldehyde **214** in 88 % after column chromatography. In this instance the alcohol and aldehyde were not crystalline.

The aldehyde was condensed with thiazolidine-2,4-dione using the Dean and Stark procedure to produce **215** in 81 % yield. It was assumed that the regio-chemistry of **215** mirrors that of **172** as they were produced in the same way and differ only in the presence of deuterium.

¹H nmr of **213-215** did not show any signs of the presence of non-deuterated compound. The level of deuteration in **215** was calculated to be 98.6 % by mass spectrometry.

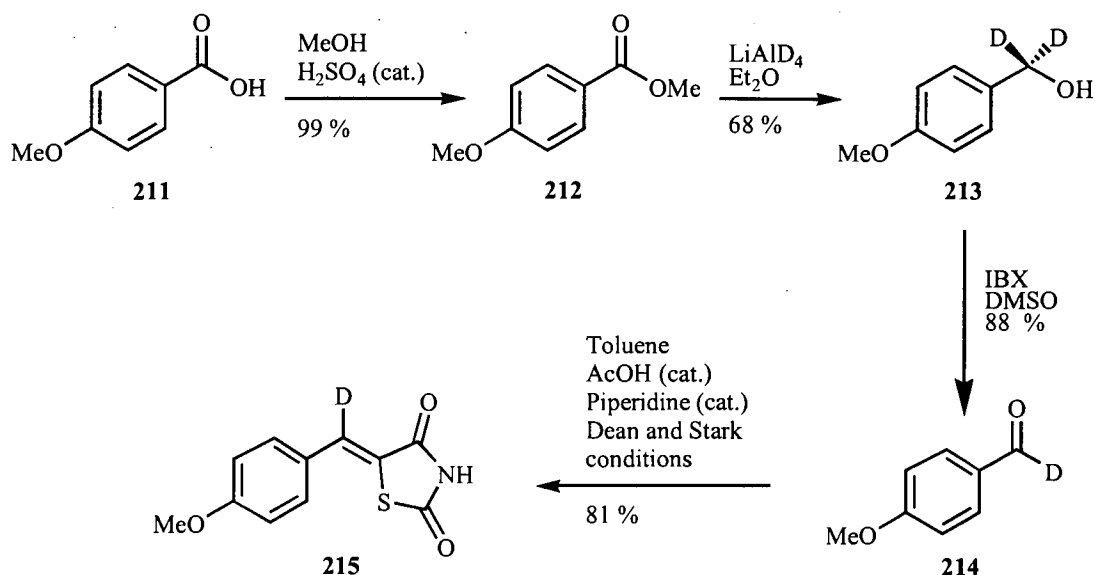


Fig. 2-6 : Synthetic Route to (Z)-5-(4-Methoxybenzylidene-d)thiazolidine-2,4-dione **143**.

2.2.2 Synthesis of 4-Propyl and 4-Butylbenzaldehyde, **216** and **217**.

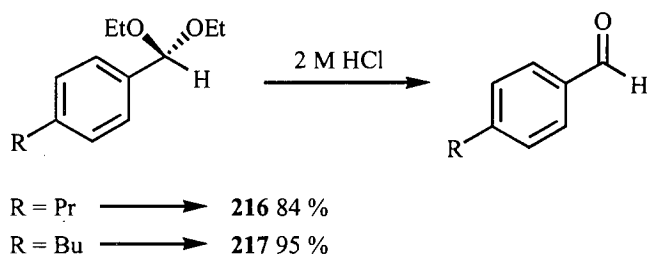


Fig. 2-7 : Deprotection of 4-Propyl and 4-Butylbenzaldehyde Diethyl Acetal.

Aldehydes **216** and **217** were both prepared by deprotection of the corresponding diethyl acetals, by stirring for one hour at ambient temperature in 2 M hydrochloric acid, in 84 and 95 % respectively (Fig. 2-7).

Attempted acetal cleavage to produce **216** with wet silica gel in DCM and sodium acetate in aqueous acetic acid proved unsuccessful.^{146,147}

Both aldehydes were condensed with thiazolidine-2,4-dione in good yield (Sect. 2.1.1).

2.2.3 Synthesis of 4-*i*-Propoxy and 4-*i*-Butoxy Benzaldehyde, **218** and **219**.

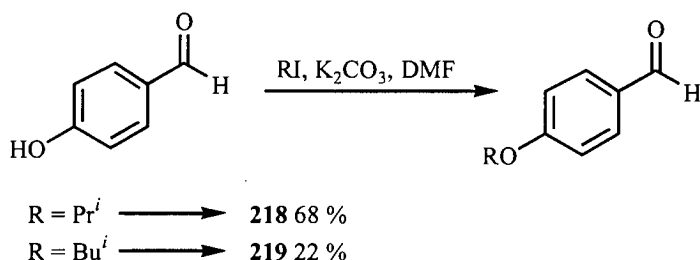


Fig. 2-8 : Protection of 4-Hydroxybenzaldehyde.

Aldehydes **218** and **219** were both produced by heating 4-hydroxybenzaldehyde and 1.1 equivalents of 2-iodopropane and 1-iodo-2-methylpropane respectively, with 1.1 equivalents of potassium carbonate, in DMF at 55 °C. After 4 and 5 d, respectively, starting material still remained which was removed by column chromatography to produce **218** and **219** in 68 and 22 % respectively.

The procedure was adapted from that used by Sargent in the synthesis of Psoromic acid and Juncunone.^{148,149} Although the yields, particularly of **219**, were poor, enough aldehyde was produced for use in the Knoevenagel condensation with thiazolidine-2,4-dione to produce **176** and **177** (Sect. 2.1.1).

2.2.4 Synthesis of 4-Benzylbenzaldehyde **222**.

4-Benzylbenzaldehyde **222** was prepared in two steps from 4-bromobenzophenone **220** via 4-benzyl bromobenzene **221** (Fig. 2-9) according to the methods utilised by Gribble to produce the same compound.¹⁵⁰

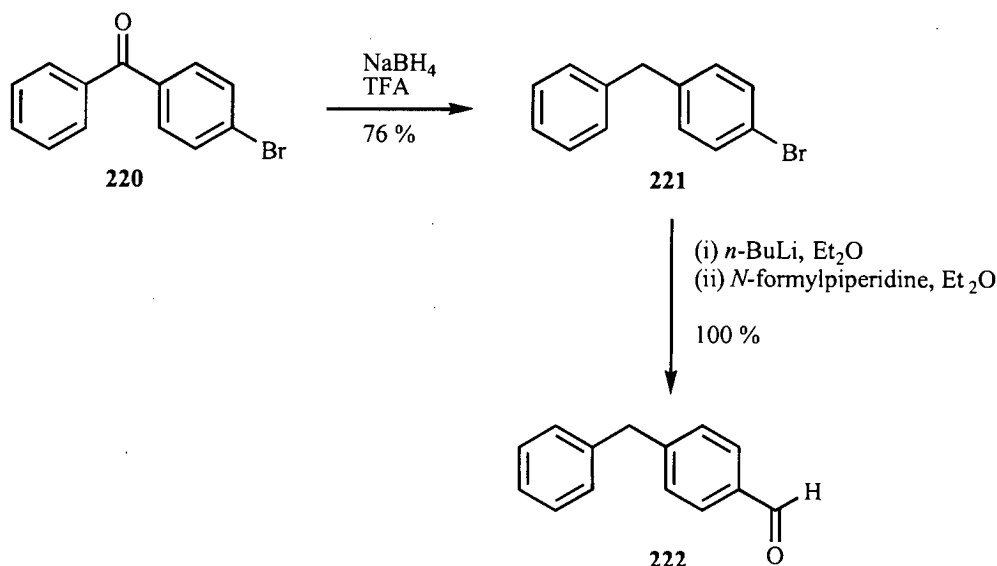


Fig. 2-9 : Synthesis of 4-Benzylbenzaldehyde **222**.

The first step involved the reduction of the ketone of 4-bromobenzophenone with six equivalents of sodium borohydride in trifluoroacetic acid to produce **221** in 76 %.¹⁵¹ The second step involved initial metal-halogen exchange between the bromine of **221** and the lithium from *n*-butyl lithium to produce the aromatic anion. This was then reacted with *N*-formylpiperidine to produce the aromatic aldehyde **222** in quantitative yield.¹⁵²

222 was then condensed with thiazolidine-2,4-dione to produce **184** in 59 % (Sect. 2.1.1).

2.3 Synthesis of the Benzylidene Precursor to Englitazone and its Isomer, *R*- and *S*-231.

The benzylidene precursor to the Pfizer drug Englitazone, (*Z*)-5-[(2*R*)-benzyl-3,4-dihydro-2*H*-1-benzopyran-6-yl)methylidene]thiazolidine-2,4-dione, *R*-**231** and its enantiomer *S*-**231**, are interesting potential substrates as they allow investigation of

whether the substitution pattern on the benzene ring is accepted by the yeast and, if so, whether or not there is any preference for one enantiomer.

The preparation of these two compounds consisted of two main stages. The initial stage involved formation of the dihydrobenzopyran skeleton upon which the benzylidene compounds are based and the resolution of 2,3-dihydrobenzopyran-2-carboxylic acid ethyl ester **227** with Lipase PS to produce the (*R*)-ester and the (*S*)-acid (Fig. 2-10). The second stage involved the conversion of *R*- and *S*-**227** into *R*- and *S*-**231** (Fig. 2-11).

Formation of the skeleton was achieved by treatment of a mixture of 2-hydroxyacetophenone **223** and diethyl oxalate **224** with sodium ethoxide followed by hydrochloric acid, to produce **225** in 61-64 %.¹⁵³ Hydrogenation over palladium on activated charcoal, at elevated temperature and pressure, removed the α,β unsaturated system to produce racemic 2,3-dihydrobenzopyran-2-carboxylic acid **226** in 56-57 %.¹⁵⁴

Esterification with ethanol and sulfuric acid in DCM afforded racemic **227** in 72-90 % which was suitable for resolution using Lipase PS, from *Pseudomonas fluorescens*. This resolution was utilised by Pfizer in a reported synthesis of Englitazone, and has been used previously in the resolution of the corresponding 7-hydroxy ester.^{155,156}

The resolution was carried out in water, without organic co-solvent, with the pH being maintained at pH 7.0 by addition of 1.0 M sodium hydroxide by means of a pH autotitrator. As the reaction proceeded there was a reduction in the rate of addition of the alkali solution indicating a reduction in rate of ester hydrolysis. Extraction of the aqueous mixture after addition of 0.55 equivalents of base afforded *R*-**227** in 43 % with 97 % e.e. (by chiral gc). Acidification of the remaining aqueous fraction followed by ethyl acetate extraction afforded *S*-**226** in 37 % which, on conversion to the *S*-**227** exhibited 77 % e.e. by chiral gc.

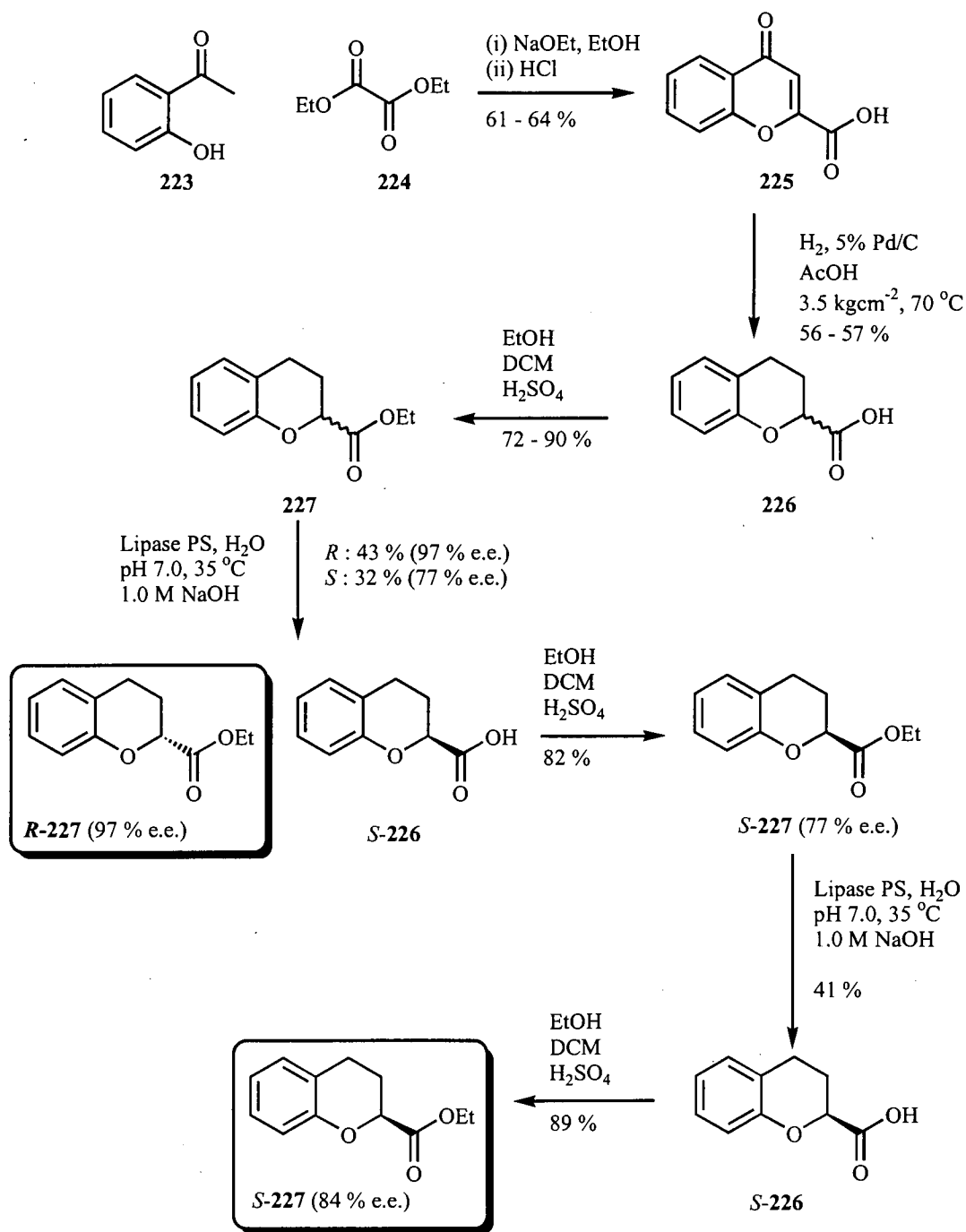


Fig. 2-10 : Synthesis of 2,3-Dihydrobenzopyran-(2R)- and (2S)-Carboxylic Acid Ethyl Ester.

A second treatment of *S*-227 increased the e.e. to 84 % on esterification, for which there is literature precedence.¹⁵⁶

Both enantiomers were converted into the benzylidene precursor to Englitzazone with relative ease (Fig. 2-11).¹⁵⁵ Initially ester **227** was reduced to the alcohol **228** by sodium borohydride in excellent yield. Each alcohol was then converted to the triflate which was displaced with phenyl magnesium bromide. Although isolated, the triflate was used immediately in the next step to produce the benzyl compounds **229** in 87-89 % (two steps). Vilsmeier formylation *para* to the cyclic heteroatom, with phosphorous oxychloride and *N*-methylformanilide, produced aldehydes *R*-**230** and *S*-**230** in 53 and 59 % respectively.

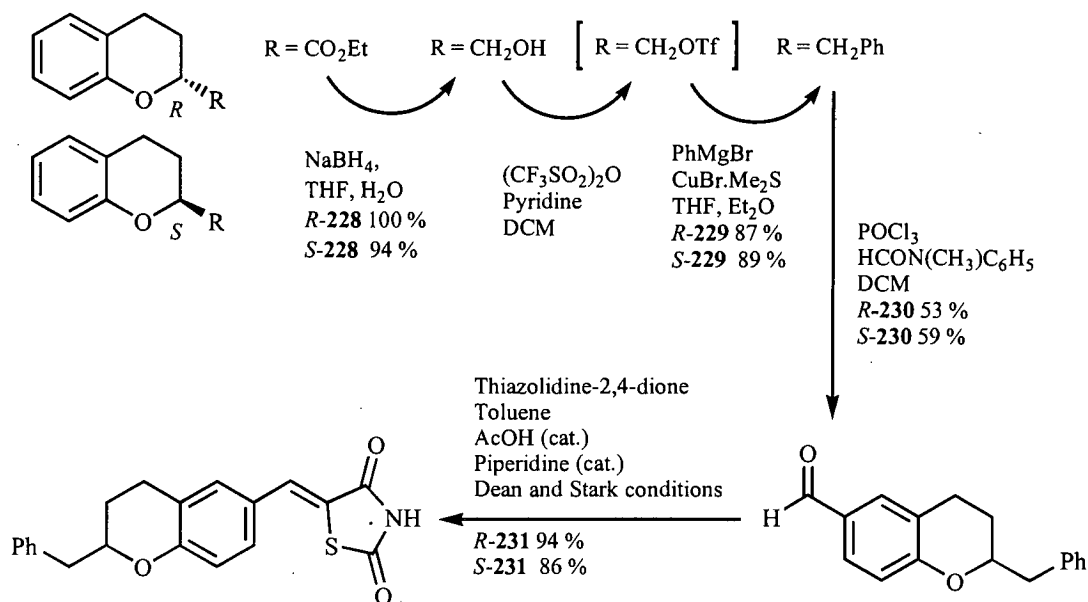


Fig. 2-11 : Conversion of **227** into **231**.

It was reported that the formylation reaction produces both *ortho* and *para* formylation products, relative to the cyclic heteroatom, and a sodium bisulfite preparation was used to isolate the *para* formylated compound. However, in the two reactions performed tlc indicated the presence of numerous compounds. In each case the use of column chromatography allowed the isolation of the *para* formylated compounds. ¹H nmr of *R*-**230** and *S*-**230** were in good agreement with the reported data.

Both aldehydes were condensed with thiazolidine-2,4-dione by heating at reflux in toluene under Dean and Stark conditions, with piperidine and acetic acid as catalyst, to produce the benzylidene compounds *R*-**231** and *S*-**231** in 94 and 86 % respectively.

¹H nmr of *R*-**231** and *S*-**231** were in good agreement with the reported data. ³J_{C,H} was measured to be 6.5 Hz for both compounds which is indicative of the *Z* isomer.

2.4 Synthesis of (Z)-5-[(2-benzoyl-5-benzofuranyl)methylidene] thiazolidine-2,4-dione 235.

235 is the unsaturated precursor to one of a class of similar saturated compounds developed through a collaboration between Tanabe Seiyaku Co. Ltd. and Eli Lilly and Co. that have been synthesised and have shown pharmacological activity towards NIDDM.¹⁵⁷ It makes an interesting potential substrate as the phenyl ring has been replaced by a benzofuran system which allows investigation of expanded substitution patterns at this end of the initial template.

Using the reported procedure 2-bromoacetophenone **232** was readily prepared by Friedel-Crafts acylation of benzene with bromoacetyl bromide in 74 % whilst 5-formylsalicylaldehyde **233** was prepared in 76 % by formylation of 4-hydroxybenzaldehyde with HMTA and TFA (Fig. 2-12). Condensation between **232** and **233** with potassium carbonate at reflux in acetonitrile proceeded readily within 22 h to produce aldehyde **234** in 56 % after washing with acetonitrile and water. Although it was reported that the reaction is complete within 3-6 h the mechanism of benzofuran formation means that disappearance of either **232** or **233** could not be used to evaluate reaction progress. To compensate the reaction was left at reflux overnight but the resultant yield is significantly less than the 84 % reported. The resulting aldehyde was condensed with thiazolidine-2,4-dione in acetonitrile with pyrrolidine as base. After heating at reflux for 20 h the crude product is removed by filtration and washed with acetonitrile to produce **235** in 63 %.

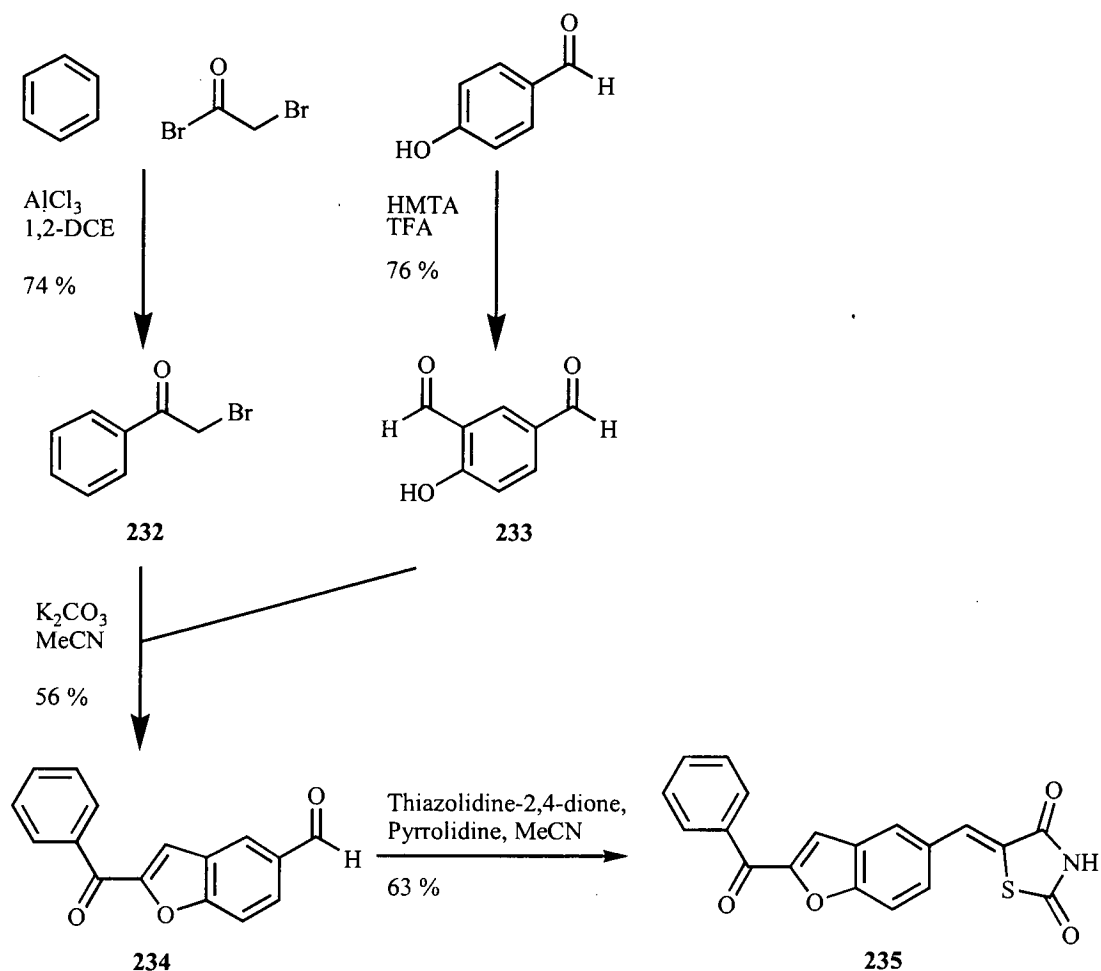


Fig. 2-12 : Synthesis of (Z)-5-[(2-benzoyl-5-benzofuranyl)methylidene]thiazolidine-2,4-dione **235**.

The reported procedure did not discuss the regio-chemistry of the double bond; however, $^3J_{\text{C,H}} = 6.5 \text{ Hz}$ which was indicative of a *cis* coupling and hence the *Z* isomer has been formed.

2.5 Expanding the Template.

Varying the substituent groups and constituent atoms at each of the five positions outlined has produced an array of compounds to probe the reductase system within the organism. However all these compounds consist of two main features, a

substituted phenyl methyldene unit and a five membered heterocycle. In order to ascertain if these are required structural features in themselves alkenes were prepared from the condensation of cyclohexane carboxaldehyde with thiazolidine-2,4-dione and by alkaline ring opening of **196**.

2.5.1 Knoevenagel Condensation Between Cyclohexane Carboxaldehyde and Thiazolidine-2,4-dione.

Replacing the phenyl ring of the template with a similarly sized aliphatic ring should help determine if the phenyl ring is required to achieve reduction of the carbon-carbon double bond.

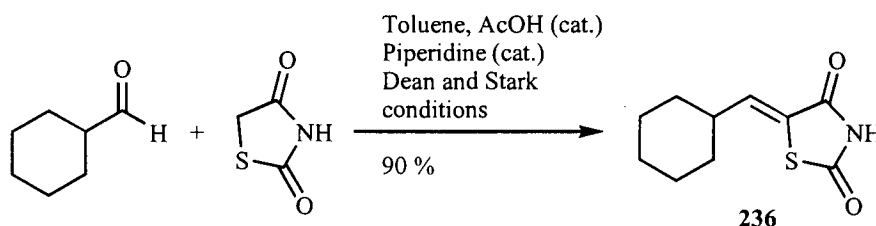


Fig. 2-13 : Knoevenagel Condensation Between Cyclohexane Carboxaldehyde and Thiazolidine-2,4-dione.

236 was synthesised using the Dean and Stark method in 90 % on purification by column chromatography (Fig. 2-13). $^3J_{\text{C,H}}$ was measured to be 6.0 Hz which is indicative of the Z isomer.

2.5.2 Ring-Opening of **196**.

The affinity of both 2-heteroatom and 2-carbon substituted 3-phenylpropanoic acids towards NIDDM has been reported by SmithKline Beecham, although the reported syntheses do not use the *Rhodotorula rubra* CBS 6469 reduction to produce these compounds from the corresponding propenoic acid.^{158,159}

All the potential substrates synthesised so far consist of a five-membered heterocycle attached to the double bond. In order to ascertain whether or not the heterocycle is an essential feature of a proposed substrate, a series of compounds based upon (Z)-2-sulfanyl-3-phenylpropenoic acid have been prepared.

These compounds were synthesised readily from **196** and retain several structural features of the parent heterocycle, namely the sulfur atom and the carbonyl at position 4 in rhodanine (Fig. 2-14).

196 can easily be converted to (Z)-2-methylsulfanyl-3-(4-methoxyphenyl)propenoic acid **238** by the procedure reported by Nishio and Ito.¹⁶⁰ This involved reaction with sodium hydroxide solution at 90 °C followed by the addition of methyl iodide. On quenching with hydrochloric acid the product precipitated from the mixture and was recovered in 59 % yield on recrystallisation from methanol, compared with the 49 % reported yield.

¹H coupled ¹³C nmr spectra of **238** showed that the ³J_{C,H} coupling between the olefinic proton and the carbonyl carbon was 6.0 Hz which is indicative of the Z geometry having been retained.

237 was prepared in a similar manner from **196**, the difference being the omission of the methyl iodide and the addition of an increased amount of hydrochloric acid, to produce the free thiol compound **237**. A similar method has been used by Ogawa in the synthesis of 2-sulfanyl-3-(3'-methyl-5-isoxazolyl)propenoic acid.¹⁶¹ Purification by washing with methanol resulted in a 94 % yield. Again ¹H coupled ¹³C nmr spectra of **237** showed the ³J_{C,H} to be 6.0 Hz, again indicating retention of the Z configuration.

Three further functional group manipulations have been carried out on **238**, the methyl and ethyl esters, **239** and **240**, were prepared by heating at reflux in DCM

with a catalytic amount of sulfuric acid and approximately five hundred fold excess of the appropriate alcohol.

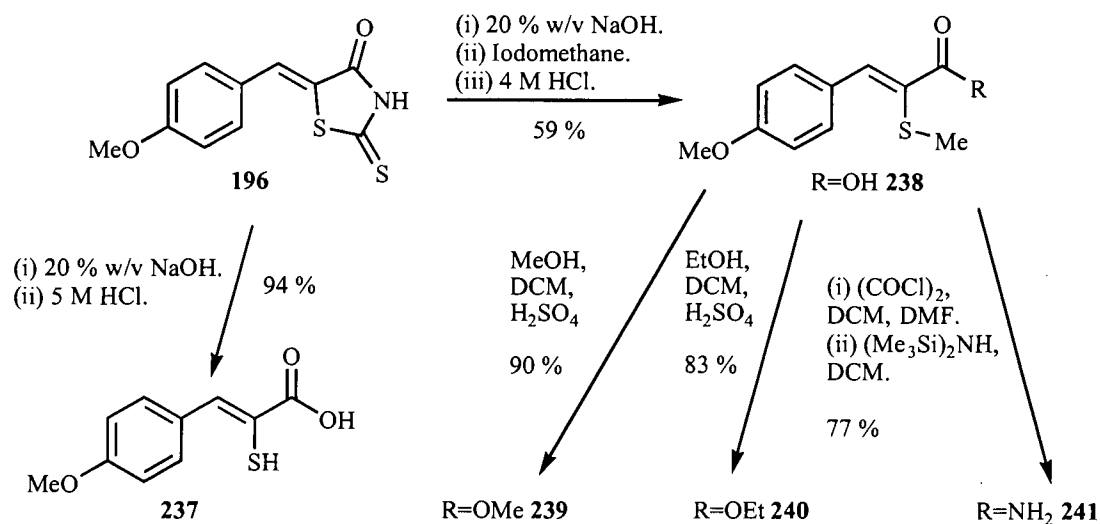


Fig. 2-14 : Synthesis of Compounds Based on 2-sulfanyl-3-phenylpropenoic acid.

The third manipulation, to produce 241, involved two steps. The first was the preparation of the acid chloride by action of oxalyl chloride and DMF on 238 according to Burgstahler's procedure, although in DCM rather than benzene.¹⁶² Although isolated the acid chloride was not characterised but instead carried through to the second procedure which involved conversion to the amide. This was achieved by stirring the crude acid chloride with 1,1,1,3,3,3-hexamethyldisilazane in anhydrous DCM for two days, according to Pellegata and Palmisano's method.¹⁶³ Both steps were carried out in an overall yield of 77 %.

As none of the procedures used to synthesise the three carboxylic acid derivatives were expected to cause isomerisation about the double bond it was assumed that all three retained the *Z* geometry.

3. Results & Discussion : Biotransformation of Substrates.

This chapter examines the activity of *Rhodotorula rubra* CBS 6469 towards the potential substrates already synthesised. Initially optimisation of the reaction will be discussed, including SmithKline Beecham work, followed by the preparative bioreduction and screening of the compounds previously synthesised.

3.1 Development of Biotransformation Conditions.

The majority of this section is based upon work by SmithKline Beecham, who have optimised the reaction conditions for the formation of BRL 49653 **2.7** Initially, various yeasts including *Candida*, *Saccharomyces* and *Pichia* spp., were screened with only the red yeasts showing significant conversion to product. Although conversions were low, *Rhodotorula rubra* CBS 6469 produced the highest conversion. The introduction of a water miscible co-solvent, 20 % v/v of ethylacetoacetate, increased the conversion of **6** to 26 %, from 13 %, after 24 h with *Rhodotorula glutinis* CBS 4406. It was found that the best conversions were exhibited when 1,4-dioxane was used as co-solvent with *Rhodotorula rubra* CBS 6469. However, there appears to be a discrepancy within the publication regarding the volume of co-solvent used. The discussion of the paper strongly advocates the use of 12 % v/v of 1,4-dioxane as the optimum co-solvent concentration, whereas the reported experimental procedure states that the substrate was added in 18.75 % v/v of 1,4-dioxane. The percentage reduction to product was found to be similar at pH 8.0 and pH 9.0 with both these exhibiting higher percentage conversions than was observed at pH 7.0. Temperature optimisation studies showed an increase of reaction rate above 24 °C with both 28 and 30 °C appearing optimal.

In addition the reported SmithKline Beecham procedure does not utilise all the cells which have been grown. Instead the cells from 90 cm³ of broth were resuspended in 69 cm³ of buffer and 40 cm³ of this was used to afford the reduction.

Initial experiments utilising the cells from 180 cm³ of broth to afford the reduction of thiazolidine-2,4-dione **172** showed that by resuspending all the cells to a total volume of 80 cm³ the rate of reaction was enhanced. These experiments also showed that the reaction rate was enhanced by use of 12 % v/v 1,4-dioxane as opposed to 18.75 % v/v. However, the rate was further enhanced by the use of DMSO, rather than 1,4-dioxane, as co-solvent (Table 3-1). DMSO was tested as a co-solvent due to the increased solubility of the benzylidene compounds, particularly those incorporating a large aliphatic chain, compared to 1,4-dioxane. Also, previous work within the group has shown that 7.5 % v/v DMF as co-solvent enhances the rate of reaction over the SmithKline Beecham procedure.¹³⁹ As the experiments were performed in an unventilated environment, DMSO was employed rather than DMF due to its lower toxicity.

Experiment 1 (Table 3-1) utilised the SmithKline Beecham conditions and involved the use of part of the total cell suspension and 1,4-dioxane (18.75 % v/v) as co-solvent. Experiment 2, while using 1,4-dioxane (18.75 % v/v) as co-solvent, utilised the whole of the biomass. Experiment 3 utilised all the cells produced and employed 1,4-dioxane (12 % v/v) as co-solvent. Finally, experiment 4 involved the use of the whole cell suspension and employed DMSO (7.5 % v/v) as co-solvent. In each case 2 cm³ aliquots of cell suspension were incubated with 150 µl of a solution of thiazolidine-2,4-dione **172** (55 mM in DMSO) to provide a control between batches of yeast. All reactions were incubated for 24 h at 30 °C and 220 rpm. In each case the average conversion measured for the two control incubations was scaled so it was equal to 100. This produced a scaling factor which, when multiplied by the conversion exhibited by the main reaction produced, a relative conversion rate. This allowed the comparison of reactions using different batches of cells.

Experiment	1	2	3	4
Volume of Growth Medium Used (cm ³)	180	180	180	180
Total Volume of Cell Suspension (cm ³)	138 ^a	84 ^b	84 ^b	84 ^b
Volume of Cell Suspension Used (cm ³)	80	80	80	80
Co-solvent Used	1,4-dioxane	1,4-dioxane	1,4-dioxane	DMSO
Volume of Co-solvent used (% v/v)	18.75	18.75	12.0	7.5
Conversion after 24 h (%)	24	49	73	58
Conversion of Control (%)	62	59	79	57
Relative Conversion	39	83	92	102

^a To provide aliquots of cell suspension for the control experiments the cells were initially resuspended to a total of 84 cm³. From this two 2 cm³ aliquots were removed. The remaining suspension was diluted to a total volume of 138 cm³.

^b The cells were resuspended to a total volume of 84 cm³. From this two 2 cm³ aliquots were removed for use in control experiments. The use of the remaining 80 cm³ constitutes use of all the cells produced.

Table 3-1 : Results of Initial Optimisation Studies with Thiazolidine-2,4-dione **172**.

Along with the reduction of thiazolidine-2,4-dione **6**, SmithKline Beecham have reported the capability of *Rhodotorula rubra* CBS 6469 to reduce the double bond of a range of related compounds **242a-f** (Fig. 3-1). However, relative conversions were not reported and each compound was added to a concentration of 0.79 mgcm⁻³ in 1,4-dioxane, although due to the difference in molecular weight of thiazolidine-2,4-diones **242a-f** this equates to between 1.9 and 2.1 mM.

The previous four reactions (Table 3-1) were all carried out on 75 mg of thiazolidine-2,4-dione **172** which equates to 3.4 mM (experiment 4) or 3.7 mM (experiments 1 and 2) depending on the resultant total volume. Use of a similar weight of thiazolidine-2,4-dione **6** in experiment 4 would equate to 2.5 mM, so in these initial reactions the concentration of substrate in the reaction has increased by a factor of approximately 1.5 over those carried out by SmithKline Beecham.

However, the use of this concentration of thiazolidine-2,4-dione **172** showed that it was possible to reduce a larger quantity of compound with the same biomass. This concentration also produced approximately 60 % conversion of **172** within 24 h which favoured the use thiazolidine-2,4-dione **172** in this concentration as a standard. It was imperative that, if relative reaction rates between compounds were to be measured, a good control was available between batches of cells and the same molar concentration of each compound was used.

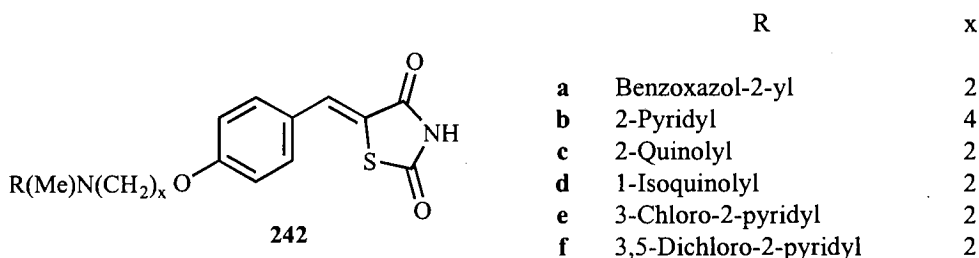


Fig. 3-1 : Benzylidene Compounds that SmithKline Beecham have Shown to be Reduced by *Rhodotorula rubra* CBS 6469.

The effect of the co-solvent concentration on the rate of reaction was determined by small scale incubations of thiazolidine-2,4-dione **172** containing various amounts of DMSO and 1,4-dioxane and is represented graphically (Fig. 3-2). The graph clearly indicates a trend of increasing relative conversion as the amount of co-solvent decreases.

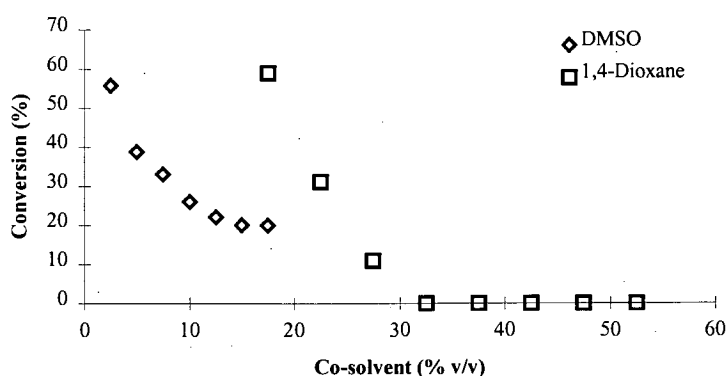


Fig. 3-2 : Graph of Co-solvent (% v/v) Against Conversion (%) for DMSO and 1,4-Dioxane.

However, experiment 4 in Table 3-1 and the value from the graph corresponding to the use of 7.5 % v/v DMSO exhibited conversions of 57 % and 33 % respectively, although the concentrations of substrate, co-solvent and biomass were the same. This difference served to highlight the problem of achieving consistency between reactions, and batches of yeast, and also the importance of standardisation using control reactions. Indeed it has been noted that as the time the organism is maintained on agar slopes increases the relative reaction rate produced by the organism decreases. Although the use of control experiments overcame this deterioration it was vital that cells from fresh agar slopes were employed in order to achieve good consistency between reactions.

Although the co-solvent concentration trend was evident, the conditions used in experiment 4 (Table 3-1) were selected for use in subsequent reactions. The use of 7.5 % v/v of DMSO guaranteed the solubility of all the potential substrates in the co-solvent. Several potential substrates were partially insoluble in the 'window' of 1,4-dioxane concentrations where conversion was exhibited.

3.2 Preparative Scale Biotransformations.

Preparative scale biotransformations were carried out on several compounds, with varying R¹ and X¹, using the conditions discussed in Sect. 3-1 (Fig. 3-3, Sect. 7.4).

Initially, several of the benzyldiene thiazolidine-2,4-diones **172**, **186**, **189** and **190** were incubated with the yeast in this manner, with all four exhibiting complete reduction and being isolated in good yield. It could also be seen that changing R¹ could have a dramatic effect on the reaction rate with the nitro and cyano groups increasing the reaction rate relative to the methoxy and the chloro.

The reduction of thiazolidine-2,4-dione **172** was monitored at intervals throughout the course of the reaction. The resultant graph shows a linear relationship between

conversion and time and is indicative of a constant rate of reaction, thus showing that product inhibition was not occurring (Fig. 3-4).

Rhodotorula rubra CBS 6469.
0.1 M Tris-HCl buffer (pH 8.0),
containing 5 % w/v sucrose, DMSO.

Substrate	R ¹	X ¹	Time (h)	Conversion (%)	Isolated Yield (%)	Product
172	OMe	S	58	100	87	243
186	Cl	S	50	100	41	244
189	CN	S	17	100	84	245
190	NO ₂	S	17	100	82	246
192	OMe	NH	164	0	-----	
193	Cl	NH	48	0	-----	

Fig. 3-3 : Result of Initial Preparative Scale Biotransformations.

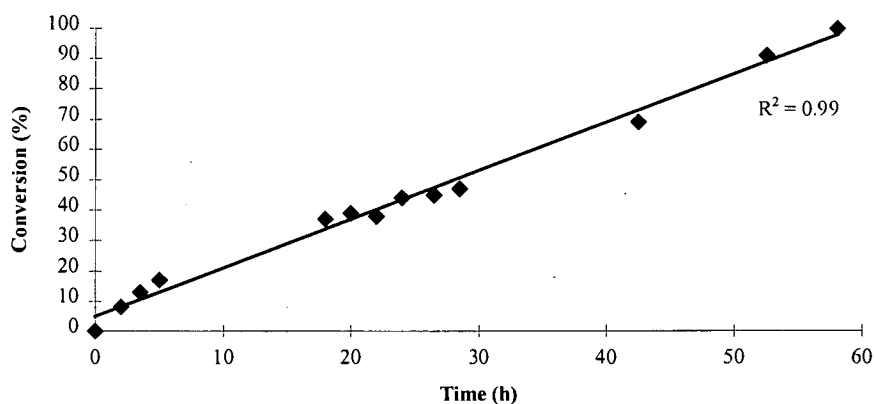


Fig. 3-4 : Conversion Against Time For *Rhodotorula rubra* CBS 6469 Mediated Reduction of 172.

It was imperative that the biotransformation achieved quantitative reduction of the benzylidene compounds as, exhibiting identical R_Fs on tlc, they could not be separated from the corresponding reduced compounds by column chromatography. As the substrates and products could not be separated by tlc, hplc was used to monitor the reactions. In the case of 172, 186 and 189 authentic samples were

produced by catalytic hydrogenation to show that separation of substrate and product was achievable under the hplc conditions (Chapter 4, Appendix 1). These hydrogenations also provided standards for the comparison of characterisation data.

Also, once complete conversion had been achieved, it was essential that product recovery was high. Ideally product isolation would have been achieved by centrifugation to remove the cells followed by a simple extraction of the supernatant with an organic solvent, though the low solubility of the compounds involved led to precipitation when centrifuged with enough force to pellet the cells. Filtration has also been shown to be an inadequate method of biomass removal hence product recovery was achieved by continuously extracting the whole biotransformation mixture into 150 cm³ of DCM for 18 h.¹⁶⁴ However, continuous extraction also removed the DMSO from the reaction mixture, which remained after evaporation of the DCM. There were two methods available to remove the product from the DMSO, column chromatography over silica gel or Kugelrohr distillation. The former was the method of choice as it also removed traces of other organic material present after the extraction, and afforded a cleaner product.

Monitoring the attempted bio-reduction of the hydantoin based compounds (**192** and **193**) by hplc did not indicate any signs of the presence of reduced compound (Fig. 3-3). In the case of **192** a standard was prepared by catalytic hydrogenation (Chapter 4) and it was shown to be possible to separate substrate and product under the hplc conditions (Appendix 1). Work up of the attempted biotransformation of **193**, using Kugelrohr distillation to ensure all organic matter was retrieved, did not produce any evidence of reduced product, by ¹H nmr or electrospray mass spectrometry. These observations showed that substitution of the ring sulfur by nitrogen stops all activity.

From these initial preparative scale biotransformations it could be seen that sulfur at X¹ appears to be essential for a potential substrate to be accepted by the reductase system, provided a useful insight into the structural features required of a substrate.

3.3 Screen of Potential Substrates Against *Rhodotorula rubra* CBS 6469.

A procedure for screening the compounds was developed which substantially decreased the time involved for the assay. This procedure involved preparing the cells in the same manner as for the preparative biotransformations (Sect. 7.5).

In carrying out this screening it was possible to derive a series of relative reaction rates. In order to do this it was essential that a comparison could be made between batches of cells. The necessity for standardisation between batches of cells was eluded to in Sect. 3.1 where consistency was achieved by performing small scale biotransformations using aliquots of cell suspension from each batch of cells grown. An alternative to this method was the standardisation of biomass from each batch using a 'dry weight curve'.

Standardisation using a 'dry weight curve' involved producing a graph of the weight of cells in a suspension against its optical density at 600 nm (Fig. 3-5). Production of this graph was achieved by suspending the cells to a total volume of 80 cm³ before various volumes were removed and diluted by a factor between 1 in 40 and 1 in 4 to a total of 20 cm³. The resulting suspension was further diluted, 1 in 25, and the absorbance was measured.

When carrying out the small scale screening and attempting to use this graph the difference in the measured optical densities was negligible. However, it has already been shown that the reaction rates produced between different batches of yeast can be quite different (Sect. 3.1). Presumably this was because the use of a 'dry weight curve' does not account for differences in the reaction conditions after substrate addition.

By reverting to comparing the conversion of a standard thiazolidine-2,4-dione compound **172** produced by an aliquot from each batch of cells it was possible to

calculate a scale factor which set the conversion of 172 arbitrarily at 100. The reaction rates of the other compound are relative to this (Table 3-2).

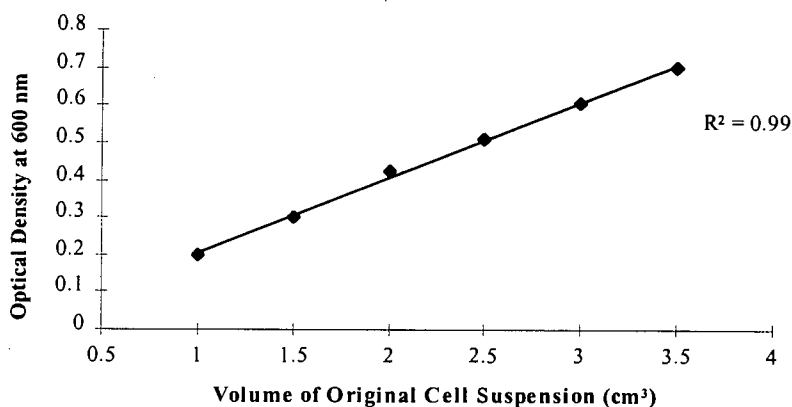


Fig. 3-5 : Volume of Original Cell Suspension v Optical Density for Rhodotorula rubra CBS 6469.

From the preparative biotransformations it has been shown that the reduced compounds exhibit shorter hplc retention times than the benzylidene compounds (Appendix 1). During the screening of compounds the presence of a peak in the hplc trace, exhibiting a shorter retention time than the benzylidene compound, established a positive result. However, failure to detect such a peak did not necessarily constitute a negative result and could be due to the fact that the reduced compound was not being separated from the starting material, or that the benzylidene compound was not being reduced.

When no such peak was detected the eluent composition was varied in an attempt to afford separation. If separation was still not achieved then an authentic sample of the reduced compound was prepared by catalytic hydrogenation (Chapter 4). If it was possible to separate this authentic sample from the benzylidene compound then it was deemed that the benzylidene compound was not a substrate. However, failure to separate this authentic sample from the benzylidene compound meant that it was not possible to determine whether or not the benzylidene compound was a substrate.

The initial piece of information that was determined from the screen results was whether or not a compound is accepted as a substrate (Table 3-2).

All compounds with a *para* alkoxy group at R¹ **172-178** were accepted as substrates as were those with a hydrogen atom **170**, hydroxy group **171** and thiomethyl group **179** in this position. However, of those with a *para* alkyl group at R¹ **180-184** only **180**, with a methyl group present, was accepted.

All four compounds with *para* halogen atoms at R¹ **185-188** were substrates, as were **189**, **190** and **191** which have a *para* cyano, nitro and trifluoromethyl group in this position respectively.

Of the compounds possessing a 2-, 3- or disubstituted aromatic ring only **198** (R¹ = 3-OMe) was reduced by the organism. However, in the case of **197** and **199** (R¹ = 2-OMe, 2,6-OMe respectively) it was not possible to tell whether or not they had been reduced by the organism during the screening process.

It could also be seen that **195** and **196** did not produce a positive result in the screen. Due to the presence of an imine and thiocarbonyl group respectively these compounds were difficult to reduce chemically (Chapter 4). However, incubation of **195** and **196**, in a manner similar to that used for the preparative biotransformations, for 48 h, followed by Kugelrohr distillation of the residual DMSO, showed that, by ¹H nmr and electrospray mass spectrometry, reduction had not occurred.

Benzylidene compounds containing a tetra-substituted double bond **201-203** did not show any signs of conversion under the screen conditions. Attempted reduction of the tetra-substituted carbon carbon double bond of ester **108**, by Santaniello and co-workers, also proved unsuccessful.⁹²

	R ¹	R ²	R ³	X ¹	X ²	Substrate	Reaction Time (h)	Conversion (%)	Conversion of 172	Scale Factor	Relative Conversion
170	H	H	H	S	O	yes	24	36	46	2.17	78
171	4-OH	H	H	S	O	yes	24	16	46	2.17	35
172	4-OMe	H	H	S	O	yes	24	46	46	2.17	100
173	4-OEt	H	H	S	O	yes	24	51	36	2.78	155
174	4-OPr	H	H	S	O	yes	24	32	46	2.17	69
175	4-OBu	H	H	S	O	yes	24	13	36	2.78	46
176	4-OPr ⁱ	H	H	S	O	yes	24	21	46	2.17	45
177	4-OBu ⁱ	H	H	S	O	yes	24	35	46	2.17	77
178	4-OBn	H	H	S	O	yes	24	6	46	2.17	12
179	4-SMe	H	H	S	O	yes	24	40	61	1.64	67
180	4-Me	H	H	S	O	yes	24	26	61	1.64	43
181	4-Et	H	H	S	O	no	24	----	----	----	----
182	4-Pr	H	H	S	O	no	24	----	----	----	----
183	4-Bu	H	H	S	O	no	24	----	----	----	----
184	4-Bn	H	H	S	O	no	24	----	----	----	----
185	4-F	H	H	S	O	yes	24	100	36	2.78	323
186	4-Cl	H	H	S	O	yes	24	88	36	2.78	244
187	4-Br	H	H	S	O	yes	24	84	74	1.35	206
188	4-I	H	H	S	O	yes	24	43	46	2.17	94

Table 3-2 : Results of Screening Potential Substrates Against Rhodotorula rubra CBS 6469

	R ¹	R ²	R ³	X ¹	X ²	Substrate	Reaction Time (h)	Conversion (%)	Conversion of 172	Scale Factor	Relative Conversion
189	4-CN	H	H	S	O	yes	6	63	25	4.00	252
190	4-NO ₂	H	H	S	O	yes	6	44	25	4.00	176
191	4-OCF ₃	H	H	S	O	yes	6	38	25	4.00	152
192	4-OMe	H	H	NH	O	no	24	---	---	---	---
193	4-OMe	H	H	NH	O	no	24	---	---	---	---
194	4-OMe	H	H	NH	O	no	24	---	---	---	---
195	4-OMe	H	H	S	NH	n.d.	24	---	---	---	---
196	4-OMe	H	H	S	S	n.d.	24	---	---	---	---
197	2-OMe	H	H	S	O	n.d.	24	---	---	---	---
198	3-OMe	H	H	S	O	yes	24	15	43	2.33	35
199	2,6-OMe	H	H	S	O	n.d.	24	---	---	---	---
200	3,5-Bu ^t -4-OH	H	H	S	O	no	24	---	---	---	---
201	H	Me	H	S	O	no	24	---	---	---	---
202	4-OMe	Me	H	S	O	no	24	---	---	---	---
203	4-OMe	Et	H	S	O	no	24	---	---	---	---
204	4-OMe	H	Me	S	O	yes	24	48	44	2.27	109
205	4-OMe	H	Bn	S	O	yes	24	49	44	2.27	111
248	4-OTBDMS	H	H	S	O	yes	24	93	46	2.17	203

Table 3-2 : Results of Screening Potential Substrates Against *Rhodotorula rubra* CBS 6469

From the preparative biotransformations it is known that the hydantoin compounds **192** and **193** do not show any reduction. This result, and the fact that **194** is not a substrate, was confirmed by the screening process. However both *N*-substituted thiazolidine-2,4-diones **204** and **205** compounds were accepted as substrates.

Other compounds were also subjected to the screening process. Neither the benzylidene precursor to Englitazone *R*-**231**, nor its enantiomer *S*-**231**, were accepted as substrates. The precursor to the Lilly drug **235** did show signs of reduction with the appearance of two new peaks in the hplc chromatogram. This was unsurprising as **235** contains a carbonyl group that could also be reduced. The reduction of **6**, the precursor to the SmithKline Beecham compound BRL 49653 **2**, was also attempted although it was not possible to tell whether or not reduction took place as **6** and **2** could not be separated under the hplc conditions.

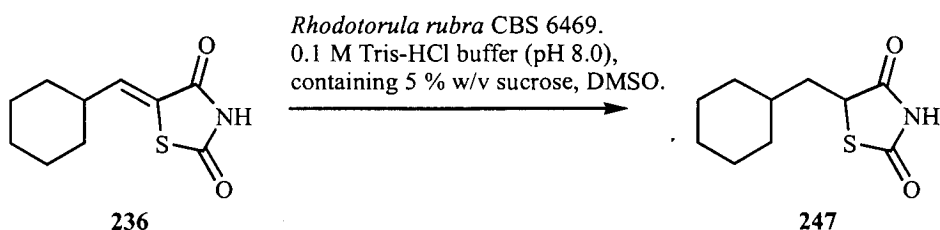
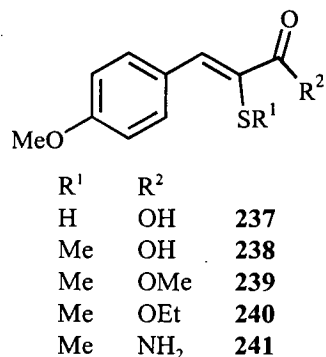


Fig. 3-6 : Rhodotorula rubra CBS 6469 Reduction of 236.

The compounds that do not conform to the initial template upon which most of the potential substrates are based were also tested. Replacement of the phenyl ring by a cyclohexyl ring **236** did not stop reduction. Unusually the screen showed formation of a compound exhibiting a longer hplc retention time than the unsaturated precursor. It was confirmed that the observed product was indeed the anticipated reduced compound by production of a standard by catalytic hydrogenation (Chapter 4). Further confirmation was obtained by yeast reduction of **236** on a preparative scale. This produced **247** in 82 % yield, after column chromatography, with quantitative conversion having been reached within 24 h (Fig. 3-6).

The range of compounds based upon (Z)-2-sulfanyl-3-phenylpropenoic acid **237-241** did not produce particularly clear hplc chromatograms which rendered the screening process non-viable for these compounds. Therefore these compounds were incubated with *Rhodotorula rubra* CBS 6469, on a preparative scale, for 48 h before being extracted. Also, as there was the possibility of the presence of hydrolytic enzymes within the yeast the residual DMSO was removed by Kugelrohr distillation to ensure retrieval of all the organic material. However, ^1H nmr and electrospray mass spectrometry showed that only starting material remained after the incubation period.



Having established which structural features were required for reduction to occur it was possible to construct a 'map' detailing these structural features, based around the initial template (Fig. 3-7).

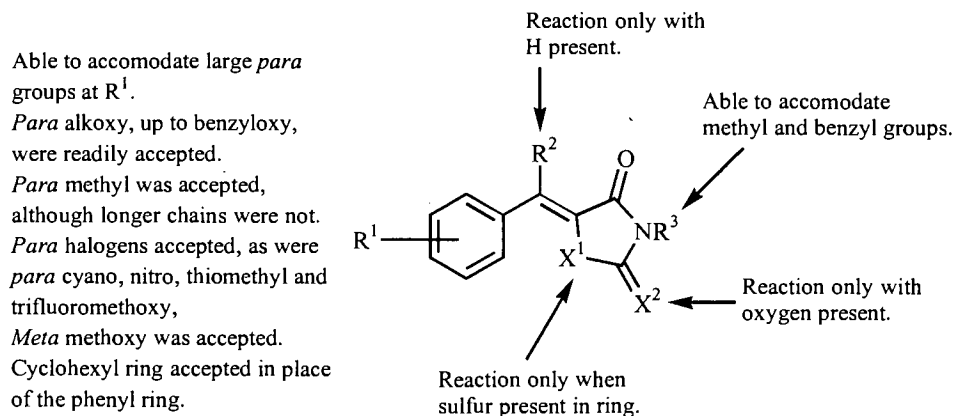


Fig. 3-7 : 'Map' Showing Structural Features Required to Effect Reduction.

There were several factors contributing to the success or failure of an attempted reduction :

1. *Uptake of Substrate.*

The ability of a substrate, or product, to diffuse through the cell wall is important. Apparently substances that are not part of the normal intracellular environment are taken up by simple (or passive) diffusion which is governed by the molecular size and lipophilic properties of the material. The cell wall does not present much of a barrier to small molecules, but it excludes molecules with a relative molecular mass greater than 600. However facilitated diffusion and active transport are also available.¹⁶⁵

2. *Active Site Fit.*

The ability of the substrate to 'fit' into the active site of the enzyme involved. As enzymes are catalysts they increase the rate of reaction by forming a complex with the substrate and lowering the energy of the transition state. The lower the energy of this transition state complex the quicker the reaction rate. If this complex cannot form then no reaction will take place.

3. *Solubility.*

Reaction rates may be increased by greater solubility of substrate.

4. *Inhibition.*

Substrates and products can have a toxic effect on the organism involved. The monitoring of the reduction of thiazolidine-2,4-dione **172** (Fig. 3-4) showed a constant rate throughout the reaction. This indicated that product inhibition was not occurring.

In the case of the longer chain *para* alkyl groups at R¹ it appeared that the size of the molecule is not the problem as similarly sized *para* alkoxy groups were accepted, as was the bulky TBDMSO group **248**. A more likely explanation is that the removal of the oxygen from the chain produced a more hydrophobic molecule which could decrease the solubility of the compound and impede its reduction. It would also be

expected that the *para* halogens **185-188**, cyano **189**, nitro **190** and trifluoromethoxy groups **191** were more hydrophilic than the long chain alkyl compounds.

Similarly, the fact that R² had to be hydrogen could stem from a solubility or diffusion problem or the inability of the enzyme's active site to accommodate a larger group in this position. The inability of the organism to reduce tetra-substituted double bonds could also be mechanistically relevant. On the other hand it is clearly possible to accommodate a larger group at R³.

These results also showed that it was necessary to have sulfur and oxygen present as X¹ and X² respectively but again this could be a result of any of the above factors.

Clearly, from these results, it is impossible to implicate any one of these effects when accounting for the success or failure of a reaction. However, it can be said that under these conditions the substrate required the structural features highlighted in Fig. 3-7 if reduction was to occur.

The nature of the screening process allowed the calculation of relative reaction rates for each of the substrates (Table 3-2). These were obtained by measuring the conversion of each substrate by hplc and scaling them against the conversion of **172** as a standard.

Obviously an error is introduced in this process. In each round of screening two aliquots of cells were used to reduce **172** with the average value used to calculate the weighting factor. The largest variation between the two **172** conversions was measured in the screening of **187** where the average of 74 % was a result of measured conversions of 70 and 78 %. Using the scale factor associated with a standard conversion of 74 % the relative reaction rate of **187** was calculated as 114; however, the maximum and minimum values, based on 78 and 70 % respectively, were 120 and 108. This gave an estimation of the error involved in the process as approximately ± 5 %.

Fig. 3-8 shows the relative conversion rates of substrates with *para* substitution at R¹. Although the values plotted are subject to a $\pm 5\%$ error there are still clear patterns present.

The alkoxy series of compounds exhibited an interesting pattern. The factors contributing to whether or not a compound was accepted as a substrate also affect the relative rate at which the compounds that are accepted were reduced. In the case of the alkoxy series it could be seen that the rate decreases on introduction of a hydroxy group (171 versus 170). The rate continued to increase through methoxy 172 and ethoxy 173 before decreasing through propyloxy 174, butyloxy 175 and benzyloxy 178. Based on hydrophobicity of the compounds alone, which would be expected to increase with increasing chain length, it was expected that the rate would decrease as the chain length increased. This is clearly not the case and other factors, such as active site fit must also be contributing.

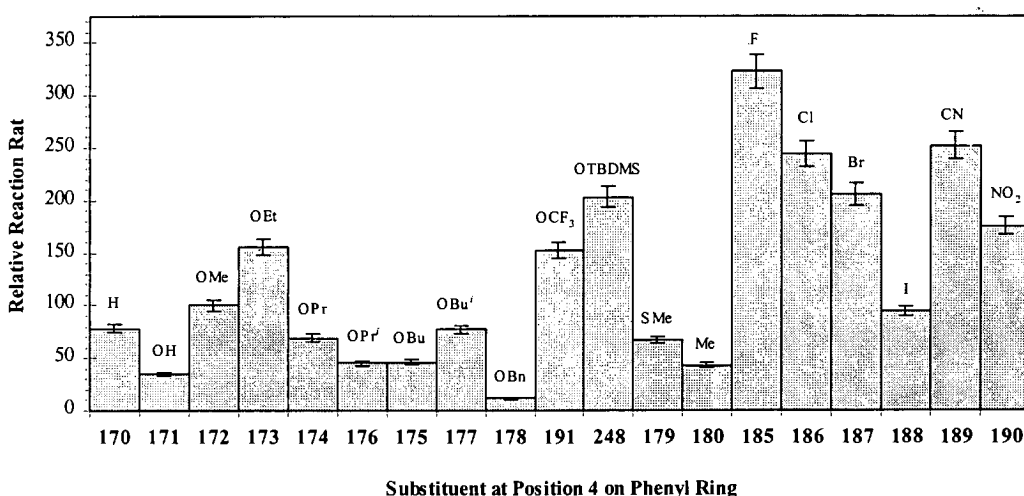


Fig. 3-8 : Graphical Representation of the Effect of Substitution at Position 4 on Relative Reduction Rate.

It could also be seen that the rate decreases along the series : 4-trifluoromethoxy 191; 4-methoxy 172; 4-thiomethyl 179 and 4-methyl 180. Although all substituents were a similar size, there is a large difference in the electronegativity of the varying substituents with the reaction rate dropping in line with this electronegativity. It

could also be seen that the polar nitro **190** and cyano groups **189** exhibited large relative reduction rates.

All four halogen compounds were reduced, with the more electronegative fluorine **185** giving rise to the largest relative reduction rate. Again this varying rate could be due to the increased polarity of the compound or could be due to the decrease of size relative to the iodo compound **188**. The variation in the physical size of the substrates does not appear to be crucial as **248**, incorporating a large TBDMSO group, was readily accepted and reduced at a relatively high rate.

The relative reaction rates have been calculated for three other types of compound which so far have not been mentioned (Fig. 3-9). These compounds include **198** where a *meta* methoxy group was incorporated at R¹. It was seen that the switching of position from *para* to *meta* drastically reduced the reaction rate. This rate reduction could have been as a result of the active site being less accommodating to substitution at this position.

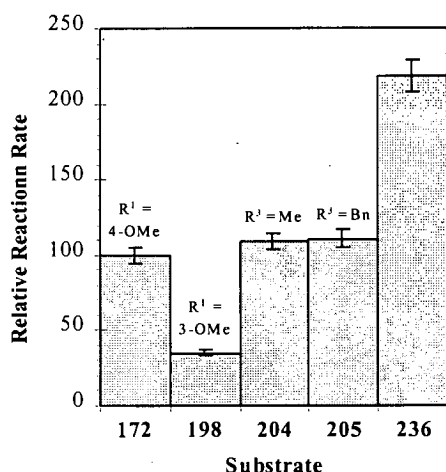


Fig. 3-9 : Relative Reduction Rates.

204 and **205** have substitution, on the ring nitrogen, at R³ in the form of a methyl and benzyl group respectively. Incorporation of these groups did not really affect the rate of reduction. This was surprising, particularly as it was expected that the hydrophilicity of **205** would differ greatly from that of **172**, and may well have been due to the difference of accommodation by the active site.

236, where the phenyl ring has been replaced by a cyclohexyl ring, exhibited a rate twice as fast as **172** and almost three times quicker than the unsubstituted aromatic

ring **170**. This rate increase could have been due to the greater flexibility of the cyclohexyl ring allowing easier diffusion through the cell membrane and easier incorporation into the active site.

It was also envisaged that there may be a relationship between the polarity of the double bond and the relative reduction rate. However, calculation of the electron densities of several substrates using CAChe v3.0 showed no such correlation. For example the electron density of the double bond of **190** was calculated as +0.006 (β position) and -0.444 (α position) whilst a similar calculation for **248** found +0.108 (β position) and -0.365 (α position). Although there is a marked difference in the polarisation of the double bonds of **190** and **248** they exhibit similar relative reduction rates (Table 3-2, Fig. 3-8).

3.4 Biotransformations With Dried and Immobilised *Rhodotorula rubra* CBS 6469.

There are certain advantages associated with the use of dried cells, which appear in two forms. The popularity of baker's yeast as a biocatalyst stems in part from the availability of the organism as a lyophilised preparation. A similar preparation can be achieved by dehydrating the organism with cold acetone to produce an 'acetone powder'.

It is also possible to entrap the organism within a polymer matrix. This can improve purification after reaction as the cells usually in the form of beads, can easily be recovered by filtration and re-use of these beads is possible.¹⁶⁶

3.4.1 Lyophilisation of *Rhodotorula rubra* CBS 6469.

Several attempts were made to utilise freeze-dried *Rhodotorula rubra* CBS 6469. Initial efforts focused on the use of cells lyophilised from 0.1 M Tris-HCl buffer (pH 8.0), containing 5 % w/v sucrose. The lyophilised cells were reconstituted by addition of water but did not afford any conversion of thiazolidine-2,4-dione **172**.

It was envisaged that as the buffer solution became more concentrated the pH may change leading to a deactivation of the enzyme system. It was then attempted to lyophilise the cells from water. Five portions of the lyophilised cells prepared from water were resuspended in 2 cm³ 0.1 M Tris-HCl buffer (pH 8.0), containing 5 % w/v sucrose, to a concentration of 50 mgcm⁻³. Each suspension was incubated at 30 °C and 220 rpm for between 0 and 5 h before 150 µl of a 55 mM solution of thiazolidine-2,4-dione **172** in DMSO was added. All were incubated for a further 16 h under the same conditions but none afforded reduction of the substrate.

3.4.2 Preparation of an Acetone Powder of *Rhodotorula rubra* CBS 6469.

The majority of acetone powder preparations in the literature are from animal sources. However, Nakamura *et al*, reported the reduction of ketones with the acetone powder of *Geotrichum candidum* in 1996 which was later used by Fujisawa *et al.*, to afford (*S*)-alcohols from (trifluoroacetyl)biphenyl derivatives.^{167,168}

Rhodotorula rubra CBS 6469 was grown and the cells produced were washed with acetone (-20 °C). This was performed twice with the yeast being left in the acetone for 5 and 10 min. The powdered yeast was resuspended in 10 cm³ 0.1 M Tris-HCl buffer (pH 8.0), containing 5 % w/v sucrose, to a concentration of 25 mgcm⁻³. 1.5 cm³ of a 117 mM solution of thiazolidine-2,4-dione **172** in DMSO was added. In each case no product was detected by hplc.

3.4.3 Immobilisation by Gel Entrapment in Calcium Alginate.

Immobilisation by entrapment within a polymer matrix is possible with a variety of polymers. In the case of polyacrylamide the polymerisation reaction between acrylamide and *bis*-acrylamide is carried out with the cells present but the monomers involved can have a toxic effect on viable cells. Entrapment procedures using κ -carrageenan and agar suffer from the drawback that the procedure is carried out at elevated temperatures. Calcium alginate is the method of choice for the entrapment of living cells as the procedure is carried out under mild conditions. This polymer is the major structural polysaccharide of marine brown algae, and the entrapment process involves the replacement of monovalent sodium ions by divalent calcium cations which leads to gelation of the polymer.¹⁶⁹

SmithKline Beecham have reported a procedure for the reduction of 6 to 2 by *Rhodotorula rubra* CBS 6469 entrapped in calcium alginate beads.⁷ The procedure is described in detail except for the type and source of the sodium alginate used, along with details of how the beads were formed. Instead it states that “the cells were immobilised into beads by standard methodology” and cites an article by Smidsrød and Skjåk-Bræk.¹⁷⁰ In addition the reported reduction only affects 87 % conversion to product.

In any entrapment of cells there has to be a balance between the rigidity of the polymer in order to prevent leakage of cells from the beads and the diffusion properties of the matrix, both of which stem from the degree of cross-linking of the polymer. The success of the procedure greatly depends upon the sodium alginate used, as increasing the concentration of alginate results in a higher degree of cross-linking within the matrix. Also, alginates constitute a family of unbranched copolymers, of 1-4 linked β -D-mannuronic acid and α -L-guluronic acid, of widely varying structure and sequence depending on their source.

The entrapment procedure results in formation of beads, the size of which are crucial in order to develop a reproducible procedure. The production of smaller beads leads to an increase in surface area which aids diffusion, the rate limiting factor in single step reactions.¹⁶⁹

If the development of a reproducible protocol for immobilisation of *Rhodotorula rubra* CBS 6469 led to the cells being reusable it could greatly reduce the time spent growing the organism making the process less labour intensive.

The calcium alginate entrapment was attempted by growing the organism in medium, washing with 0.1 M Tris-HCl (pH 8.0), containing 5 % w/v sucrose, before resuspending in the same buffer system. This suspension was mixed with a 2 % w/v solution of medium viscosity sodium alginate in the same buffer in a 1.0 : 1.0 ratio. The mixture was extruded from a syringe into 0.2 M aqueous calcium chloride and allowed to harden for 25 min before being removed and thoroughly washed with water. The beads were then resuspended in the same buffer and thiazolidine-2,4-dione **172** (5.0 mM) was added in 1,4-dioxane (18.75 % v/v). This resulted in 20 % conversion to product after 24 h which remained constant.

By utilising low viscosity sodium alginate in an identical procedure the conversion was increased to 97 %. The scale of the reaction was then increased, with the organism being grown in twice the volume of medium, and the ratio of cell suspension to alginate solution altered. The cells were resuspended and mixed with a 2 % w/v solution of low viscosity sodium alginate solution in buffer in a 1.2 : 1.0 ratio. Again the beads were formed by extrusion into 0.2 M aqueous calcium chloride. By adding thiazolidine-2,4-dione **172** (3.7 mM) in DMSO (7.5 % v/v) to the resuspended beads it was possible to achieve quantitative conversion within 44-48 h and isolate the product in 58 % yield. Quantitative conversion with the free cells was achieved in 58 h; however, there were no systems in place to compare conversion between the two methods.

Although the beads were easily removed by filtration the resulting solution appeared slightly cloudy. This cloudiness was evidence of leakage from the beads, and indeed streaking of the filtrate on agar plates showed the presence of viable cells. This is problematic as it led to the formation of emulsions when attempting to extract the product and therefore it is necessary to continuously extract the product into DCM.

An attempt was made to re-use the polymer beads but the second attempt at reduction of **172** reached a maximum value of 75 % conversion after 44.5 h. This inability to re-use the alginate beads, and the failure to simplify the purification procedure, meant that, without further development, the use of the immobilised cells would not have greatly improved the overall procedure for performing preparative scale reductions.

4. Results & Discussion : Chemical Reduction.

In investigating the biotransformation of the substrates it was necessary to produce several reduced compounds as standards which were mainly used to compare hplc retention times. This was of particular importance during the screening of compounds. If there was no sign of product by hplc it was essential to show that the starting material and anticipated product could be separated under the hplc conditions before it could be demonstrated that the benzylidene compound was not a substrate.

4.1 Reduction of Tri-substituted Double Bonds.

4.1.1 Hydrogenation.

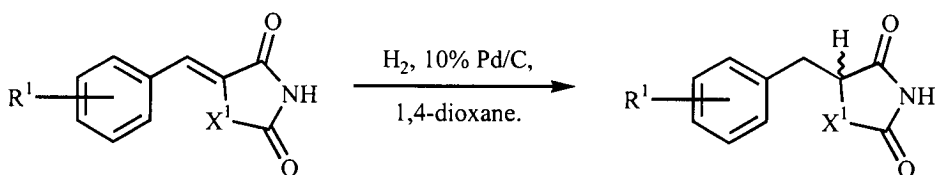


Fig. 4-1 : Hydrogenation of Tri-substituted Double Bonds.

For the majority of tri-substituted alkenes that required reduction it was possible to achieve this by hydrogenation over 10 % Pd/C in 1,4-dioxane (Table 4-1, Fig. 4-1). Three different methods were employed with more catalyst being required when X¹ is sulfur. In the main yields were high with the notable exception of the 4-CN compounds **245** (29 %) and **255** (45 %). In each case it is possible that reduction of the nitrile functionality could have occurred to a certain extent. If nitrile reduction was occurring then the resultant polar amine would have been removed during column chromatography. In the production of **245** the low yield can partially be

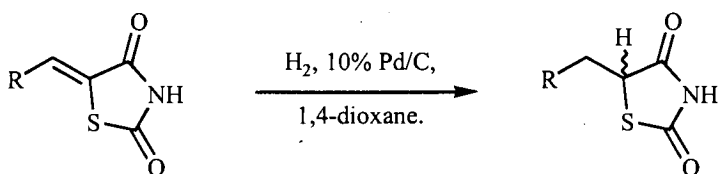
Starting Material	R ¹	X ¹	Concentration of Starting Material (mM)	Concentration of 10 % Pd/C (mgcm ⁻³)	Ratio of Starting Material to 10 % Pd/C (mmol g ⁻¹)	Time (h)	Yield (%)	Product
172	4-OMe	S	44	20	2.2	24	75	243
180	4-Me	S	44	20	2.2	24	100	249
181	4-Et	S	45.5	20	2.3	30	68	250
182	4-Pr	S	44	20	2.2	24	79	251
183	4-Bu	S	44	20	2.2	24	75	252
184	4-Bn	S	44	20	2.2	24	57	253
186	4-Cl	S	44	20	2.2	24	53	244
189	4-CN	S	45.5	20	2.3	24	29	245
192	4-OMe	NH	44	2.3	19.1	18	90	254
194	4-CN	NH	44	2.3	19.1	24	45	255
197	2-OMe	S	45.5	20	2.3	46	83	256
199	2,6-OMe	S	45.5	20	2.3	46	79	257
200	3,5-Bu ^t -4-OH	S	45.5	20	2.3	48	87	258

Table 4-1 : Results of Hydrogenation of Tri-substituted Alkenes Over 10 % Pd/C.

attributed to the loss of insoluble material during removal of the catalyst by filtration through Celite™.

Hydrogenation of **190** ($R^1 = \text{NO}_2$, $X^1 = \text{S}$) under similar conditions produced a complex mixture of products from which nothing of interest could be extracted.

Three other compounds have all been reduced in this manner to produce **259** (56 %), **2** (64 %) and **247** (78 %) respectively (Fig. 4-2).

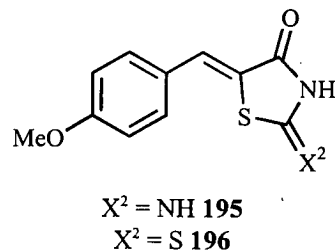


Starting Material	R	Time (h)	Yield (%)	Product
<i>R</i> - 231		21	56	259
6		48	64	2
236		27	78	247

Fig. 4-2 : Results of Hydrogenation of *R*-**231**, **6** and **236** over 10 % Pd/C.

4.1.2 Other Methods.

Hydrogenation of **195** and **196** did not appear appropriate due to the presence of the imine and thiocarbonyl respectively. It has also been noted that the presence of the thiazolidine-2,4-dione ring facilitates the need to use a large proportion of catalyst, presumably due to catalyst poisoning by the sulfur atom. The presence of another sulfur atom in **196** would lead to further catalyst poisoning.



It was attempted to reduce **196** using zinc dust and acetic acid according to the procedure reported by Hansen.¹⁷¹ Although it was noted that this procedure leads to thiocarbonyl removal it was reported that material can be produced where the alkene double bond is reduced and the thiocarbonyl group left intact (Fig. 4-3).

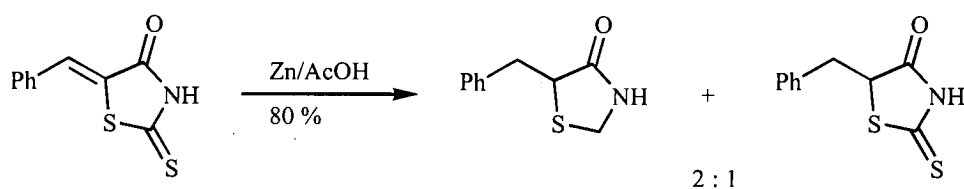
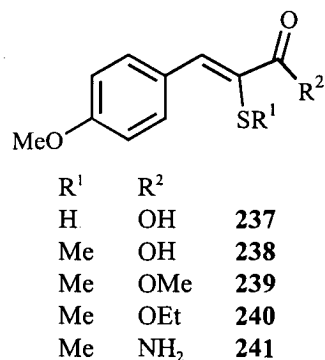


Fig. 4-3 : Reported Zinc and Acetic Acid Reduction.

Attempts to reproduce this procedure on **196** were unsuccessful as purification proved difficult because the solid crude product was not amenable to column chromatography.

Attempts to hydrogenate **237**, **238** and **240** were unsuccessful, with ¹H nmr not indicating the presence of any reduced compound.



Therefore it was attempted to reduce **239** and **241** using dissolving magnesium in methanol but ^1H nmr and electrospray mass spectrometry did not show any signs of reduced product.⁷

Also, attempted reduction of **195** and **196** with magnesium in methanol did not reduce the carbon carbon double bond.

4.2 Reduction of Tetra-substituted Double Bonds.

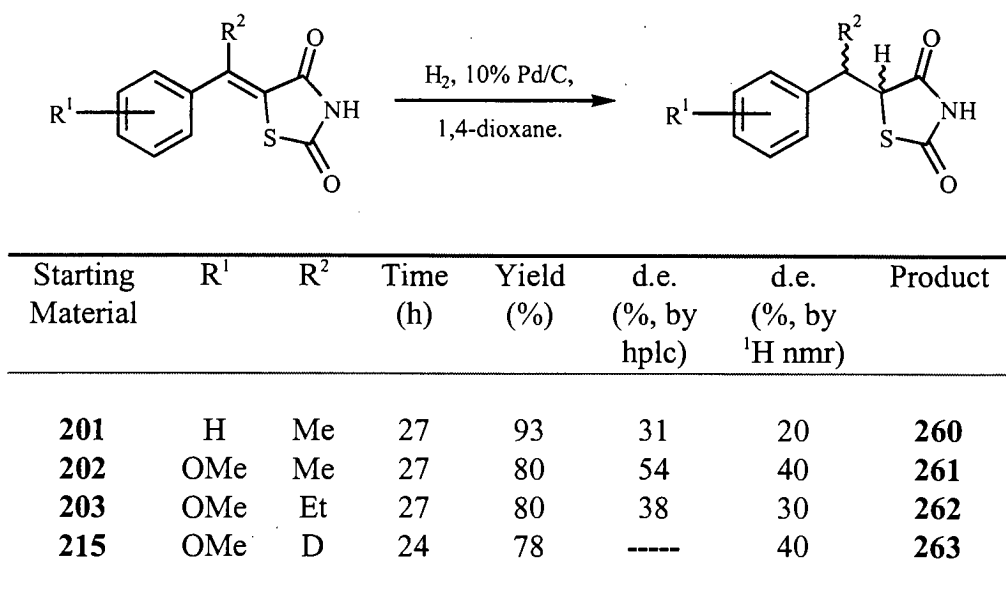


Fig. 4-4 : Results of Hydrogenation of Tetra-substituted Alkenes over 10 % Pd/C.

As the three tetra-substituted alkenes **201-203** did not appear to produce any reduced compound on incubation with the yeast they were hydrogenated over 10 % Pd/C in 1,4-dioxane (Fig. 4-4). All three products exhibit an excess of one diastereomer, as does **263**.

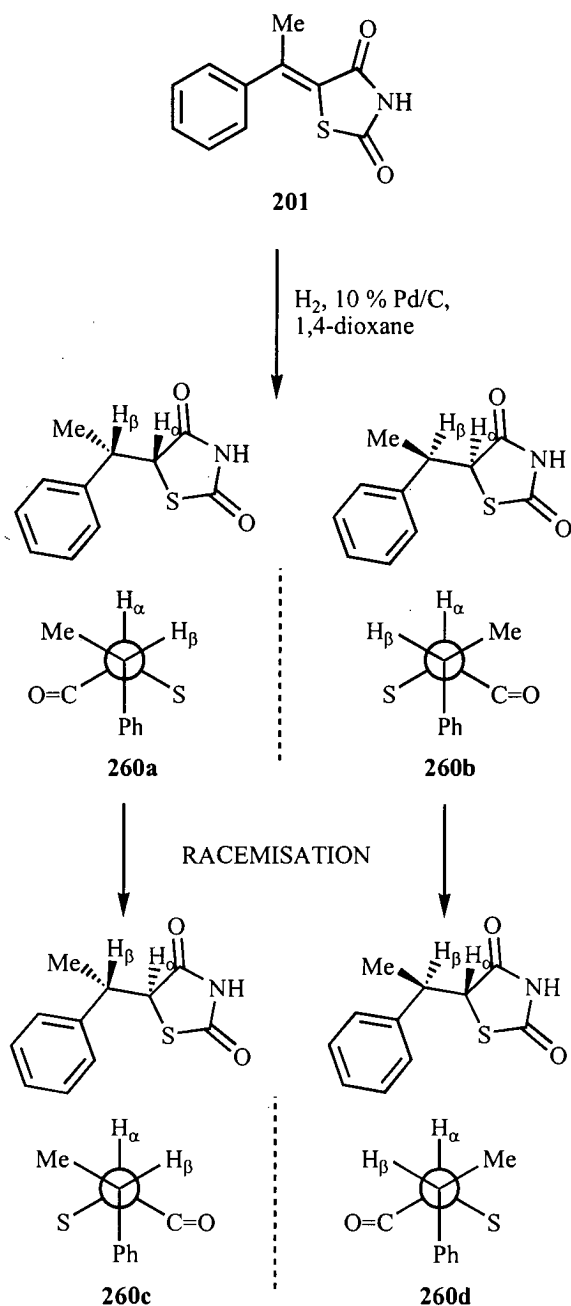


Fig. 4-5 : Proposed Mechanism for the Formation of Two Diastereomers in the Hydrogenation of **201**.

This diastereomeric excess is a peculiar result which requires some explanation. It would be expected that hydrogen addition across the double bond would produce a statistical mixture of compounds as there should be no discrimination between enantiotopic faces of the molecule.

However, when hydrogen is added statistically in a *syn* fashion two enantiomers are created (eg. **260a** and **260b**, Fig. 4-5). It is known that the centre α to the sulfur atom is prone to racemisation which would produce a further two compounds **260c** and **260d** which are enantiomers of each other but diastereomers of **260a** and **260b**.

The Newman projection formulae of **260a** and **260b** clearly show that H_α , H_β and the methyl group are in the same environment in both molecules, hence they would produce the same nmr spectrum.

Projections of **260c** and **260d** show a similar scenario.

However, on going from **260a** to **260c**, and similarly from **260b** to **260d**, it can be seen that racemisation changes the environment of H_β and the methyl group.

So, although **260a** and **260b** exhibit the same spectrum, as do **260c** and **260d**, they differ from each other.

This explanation accounts for the discrepancies in d.e. values measured by hplc and ^1H nmr for **201-203**. The data from hplc was collected first and indicates a higher d.e. than the ^1H nmr data (Fig. 4-4). It is feasible that the racemisation is slow, leading to a decrease in d.e. in the time between acquiring both sets of data. ^1H nmr's were recorded in CDCl_3 which may be slightly acidic and could increase the rate of racemisation.

This mechanism would also account for the d.e. exhibited by **263**. By following the process as outlined in Fig. 4-5 for the hydrogenation of **215** a similar scenario emerges where the methyl group of **260** is replaced by a deuterium atom. No hplc data is available for **263** as the chirality β to the sulfur arises as a result of isotope incorporation.

In each instance **260-263** the more abundant species has the smallest coupling between H_α and H_β . The reduction of **201** would be expected to produce a majority of **260a** and **260b**, with a *syn* relationship between H_α and H_β .

5. Results & Discussion : Biotransformation Mechanism.

This chapter describes work directed towards the elucidation of the mechanism of the double bond reduction using *Rhodotorula rubra* CBS 6469.

5.1 Biotransformation of (Z)-5-(4-methoxybenzylidene-d) thiazolidine-2,4-dione **215**.

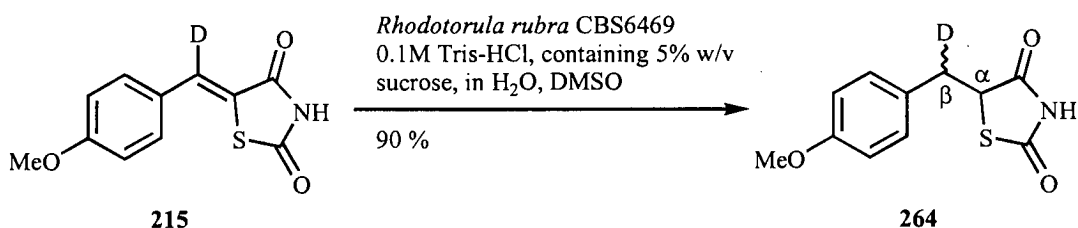


Fig. 5-1 : Biotransformation of (Z)-5-(4-methoxybenzylidene-d)thiazolidine-2,4-dione **215** in Hydrated Medium.

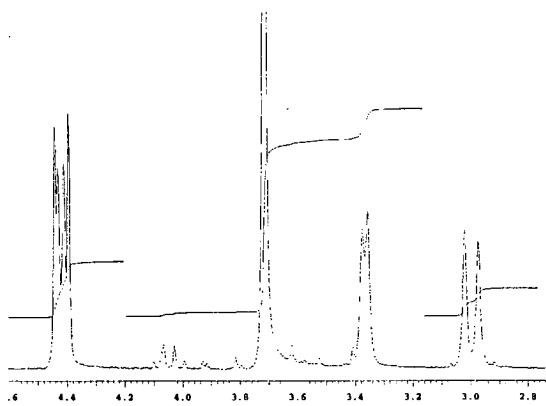


Fig. 5-2 : ¹H nmr of **264**



Fig. 5-3 : ²H nmr of **264**

The initial experiment carried out in this area involved the biotransformation of the deuterated substrate **215** (Fig. 5-1) which produced two separate products in a 1:1 ratio. A similar result was noted in the hydrogenation of **215** to produce **263**. It was proposed that the formation of two compounds in the hydrogenation of **215** resulted

from *syn* addition of hydrogen followed by racemisation α to the sulfur (Sect. 4-2). However, in the case of **264** the initial reduction product would be expected to fully racemise under the reaction conditions.

Two modes of double bond saturation, *syn* and *anti*, are possible which, after racemisation can produce **264a-d** (Fig. 5-4).

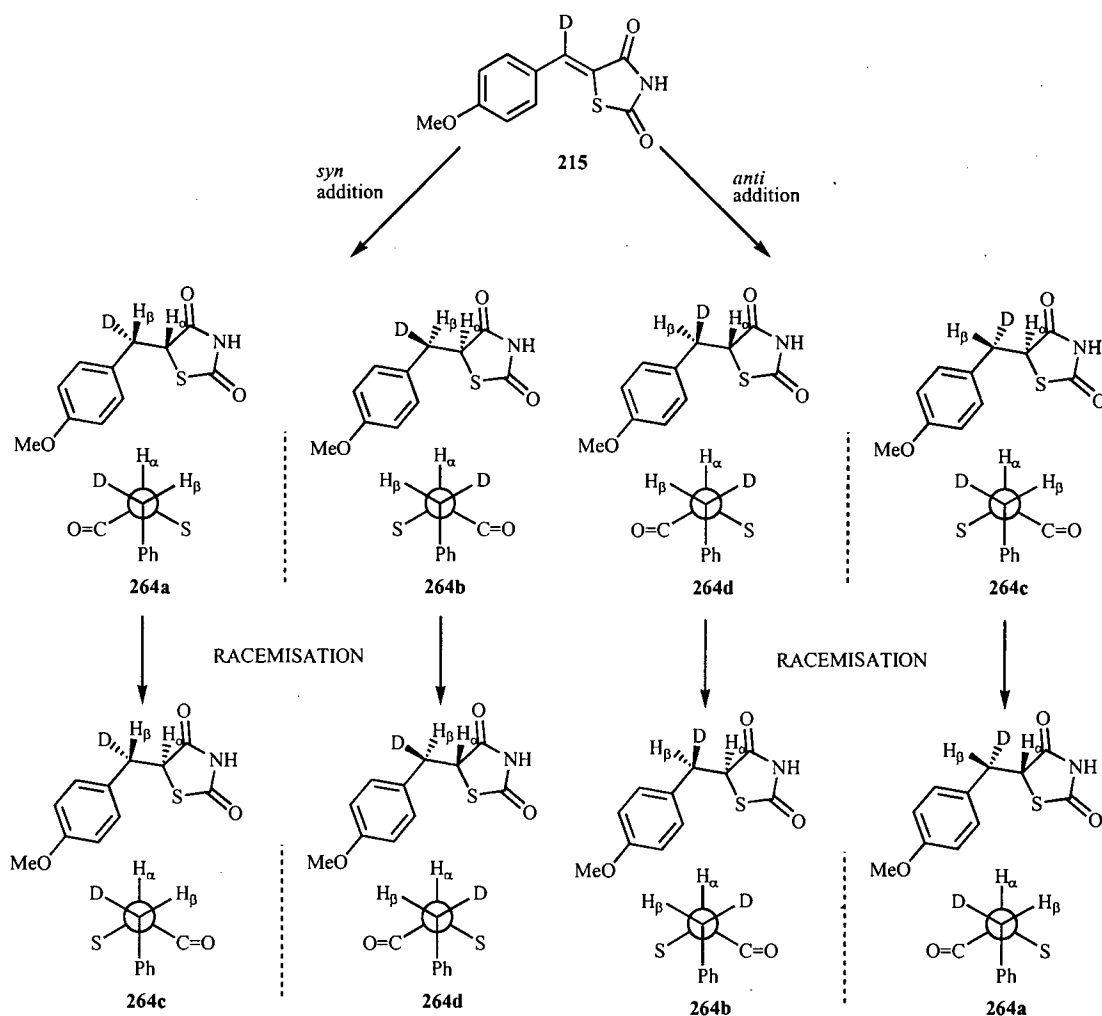


Fig. 5-4 : Possible Products From the *Rhodotorula rubra* CBS 6469 Mediated Saturation of the Carbon-Carbon Double Bond of **215**.

264a-d consists of two pairs of enantiomers as can be seen from the Newman projection formulae (dashed lines represent mirror planes). It can be seen that **264a** and **264b** can be formed from *syn* addition or from *anti* addition followed by

racemisation. Similarly **260c** and **260d** can be formed by *anti* addition or by *syn* addition followed by racemisation.

This shows that there are two chemically different species present. One with a *syn* relationship between H_α and H_β , **264a** and **264b**, and another with an *anti* relationship between H_α and H_β , **264c** and **264d**.

The two species are clearly evident in both the ^1H and ^2H nmr spectra (Figs. 5-2 and 5-3). One exhibits a doublet (J 9.5 Hz) at δ_{H} 3.07, arising from H_β , and a doublet (J 9.5 Hz) at δ_{H} 4.49, arising from H_α , whilst the other exhibits a doublet (J 4.0 Hz) at δ_{H} 3.43, arising from H_β , and a doublet (J 4.0 Hz) at δ_{H} 4.49, arising from H_α . The difference in the environment of H_β is greatly different between the two species leading to a large difference in δ_{H} , however the difference in environment of H_α is much smaller so both doublets arising from H_α appear to be almost superimposed.

Although the Newman projections show a similar dihedral angle between H_α and H_β , in both species, it has to be remembered that these are diagrammatic representations designed to aid the clarity of explanation and do not represent the exact structure of the two species. As they both display different coupling constants between H_α and H_β the dihedral angle between these two protons must differ between species.

It is impossible to tell the absolute stereochemistry of either centre and, therefore, from which face H_β is incorporated.

215 has a deuterium content of 98.6 %, as measured by low resolution mass spectrometry. A similar treatment of **264** gives no indication of the presence of non-deuterated compound indicating that no loss of deuterium has occurred.

5.2 Biotransformation of (Z)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione **172** in Deuterated Medium.

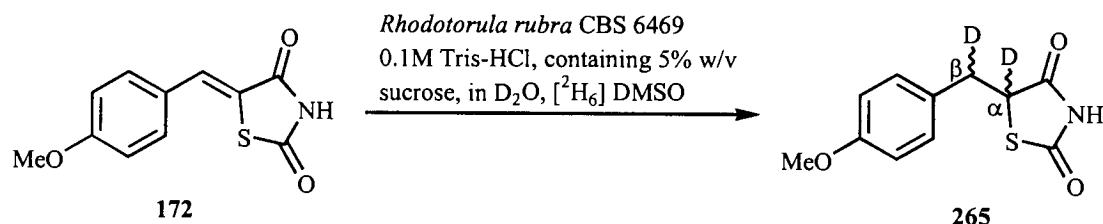


Fig. 5-5 : Biotransformation of (Z)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione **172** in Deuterated Medium.

In a complementary experiment, the reduction of **172** using *Rhodotorula rubra* CBS 6469 was conducted in deuterated buffer (Fig. 5-5).

The use of deuterated buffer dramatically reduced the rate of reaction. This reaction required seven days to effect 87 % conversion as opposed to approximately two days to reach quantitative conversion in non-deuterated medium. This is indicative of some type of deuterium isotope effect, although the exact nature of the effect is impossible to ascertain.

Examination of the ¹H nmr of **265** at 600 MHz revealed the presence of six different isotopomers in the product mixture (Fig. 5-8).

The region arising from H_α in the reduced compound at 4.49 ppm (Fig. 5-6a) indicates the presence of three species. The deuterium decoupled spectrum (Fig. 5-6b) is more informative and the four smaller peaks, labelled 1-4, exhibit the same chemical shift and coupling constants as the reference material **243** (Fig. 5-6c), prepared by catalytic hydrogenation (Chapter 4). In this case H_α is coupled to the two protons at H_β with *J* 4.0 Hz (peaks 1-2) and 9.5 Hz (peaks 1-3) (Fig. 5-8, **265**C_H).

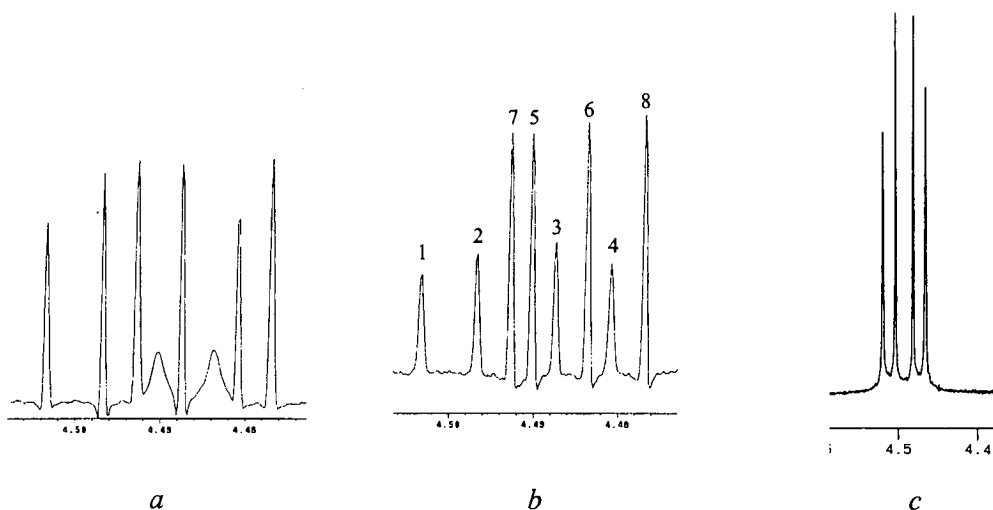


Fig. 5-6 : a. ^1H nmr Expansion; b. ^1H nmr Expansion with Deuterium Decoupling; c. ^1H nmr Expansion of **243** as Reference.

Two doublets were also present in the deuterium decoupled spectrum, one with J 4.0 Hz (peaks 5-6) and the other J 9.5 Hz (peaks 7-8), indicating that two further species are present. These species appeared to coincide with the two products present in **264**, one with a *syn* relationship between H_α and H_β (Fig. 5-8, **265A_H**) and another with an *anti* relationship between H_α and H_β (Fig. 5-8, **265B_H**). It has to be remembered that the stereochemistry portrayed for **265A_H** and **265B_H** is relative and that the enantiomers of both these compounds may also be present.

Although it is not possible to determine which doublet arises from which species it is assumed that the *syn* relationship between H_α and H_β **265A_H** gives rise to J 4.0 Hz.

The integrals across this region showed that the ratio of the three isotopomers was 1.00:1.00:1.28 (**265A_H**:**265B_H**:**265C_H**).

Further evidence for the presence of these three isotopomers is available in the two regions of the ^1H nmr spectrum arising from H_β (Fig. 5-7). The non-deuterated species **265C_H** exhibits a characteristic doublet of doublets in both of these regions, as the reference spectra (Fig. 5-7a) showed, which were identified in the deuterium decoupled spectrum of the mixture of biotransformation products (Fig. 5-7d, peaks 9-12, and Fig. 5-7e, peaks 17-20). The coupling constants of 4.0 and 14.0 Hz, in the

region at lower field, and 9.5 and 14.0 Hz, in the region at higher field, are in good accordance with those exhibited in the reference spectrum.

265A_H should exhibit a doublet with J 4.0 Hz in the lower field region whilst **265B_H** should give rise to a doublet with J 9.5 Hz in the higher field region.

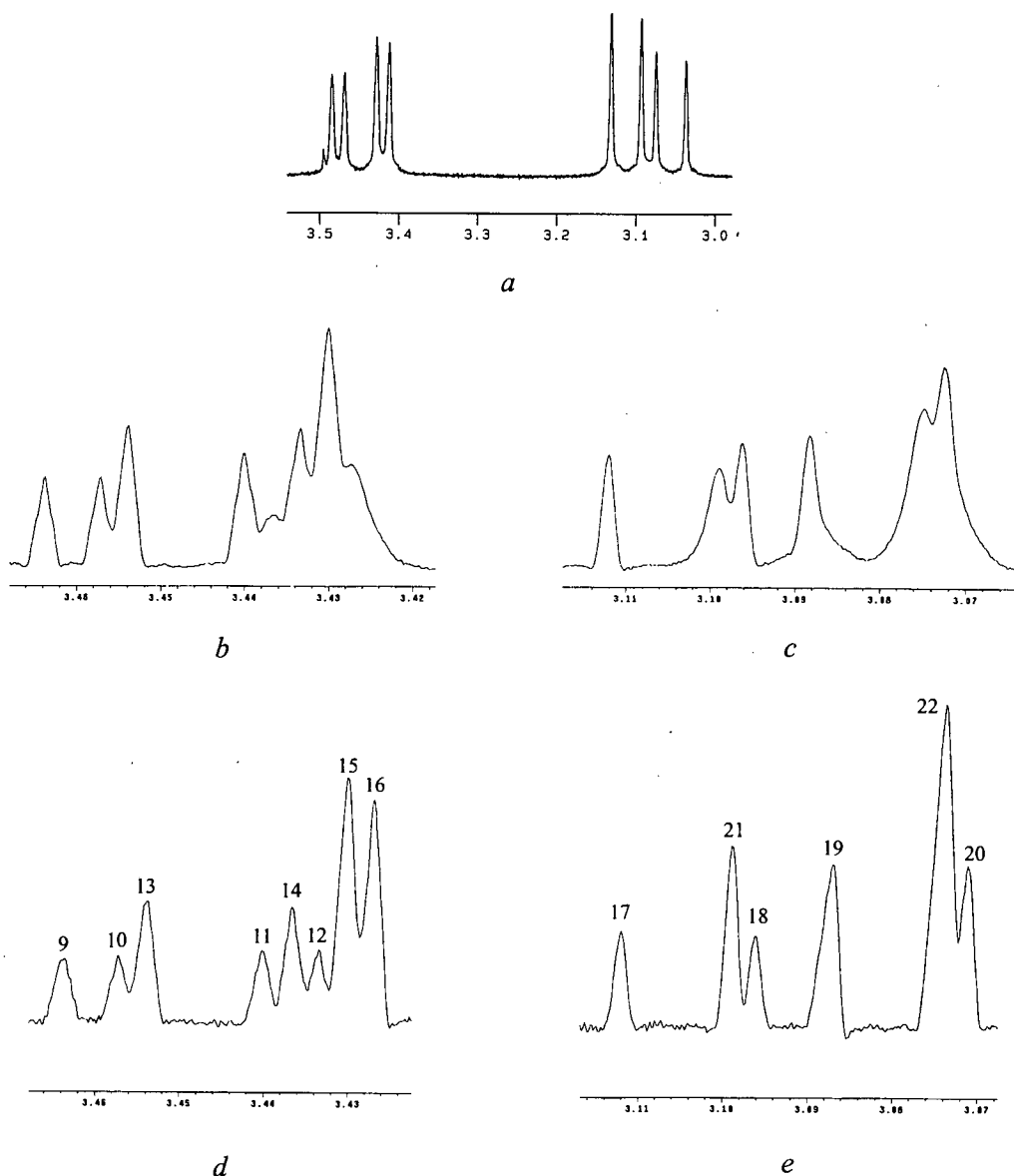


Fig. 5-7 : a. ^1H nmr Expansion of **243** as Reference.
 b. ^1H nmr Expansion of H_β at Lower Field.
 c. ^1H nmr Expansion of H_β at Higher Field.
 d. ^1H nmr Expansion of H_β at Lower Field with Deuterium Decoupling.
 e. ^1H nmr Expansion of H_β at Higher Field with Deuterium Decoupling.

Although peaks 19 and 20 are part of the doublet of doublets arising from **265C_H** their intensities were greater than expected from the 'roofing' pattern exhibited by the reference material. It is possible that these overlap with a doublet with J 9.5 Hz, whilst in the similar spectrum from the proton at lower field (Fig. 5-7d) there was a doublet with J 4.0 Hz, peaks 14-15.

It is known that the reduced product is susceptible to racemisation at pH 8 which in this case would lead to incorporation of deuterium at H _{α} and hence it is feasible that there are such compounds, corresponding to each of the three compounds identified, where deuterium has been incorporated at H _{α} .^{7,172} This racemisation would give rise to **265A_D**, **265B_D** and **265C_D** (Fig. 5-8). It is important to remember that the stereochemistry drawn for **265A_D**, **265B_D**, and **265C_D** are relative and that the enantiomer of each one may be present.

Peaks 13 and 15 in Fig. 5-7d appeared as a doublet with J 14.0 Hz. A similar pattern was exhibited between peaks 21 and 22 in Fig. 5-7e. These patterns were characteristic of coupling between the two H _{β} sites, but with no coupling to H _{α} , and were indicative of the presence of **265C_D**.

A singlet in each of the H _{β} positions was indicative of **265A_D** and **265B_D** and peak 16 (Fig. 5-7d) does not appear to couple to any of the other peaks in this region. It was possible that a similar peak occurs in the other H _{β} region, but was obscured. A similar phenomenon was seen with peaks 19 and 20 and further evidence for this was obtained by looking at the integrals across both these positions.

Each of the H _{β} regions were considered in turn, starting with that at lower field (Table 5-1). Peaks 9-12 arose from **265C_H** and each displayed the same integral, whilst peak 15 coupled to both 13 and 14 and arose from both **265C_D** and **265A_H**. The relative integral of 15 was equal to the total displayed by 13 and 14 as expected which leaves peak 16 which arose from **265A_D**. Relative ratios of these four

compounds were calculated based on the total integral exhibited by each species in this region (Table 5-2).

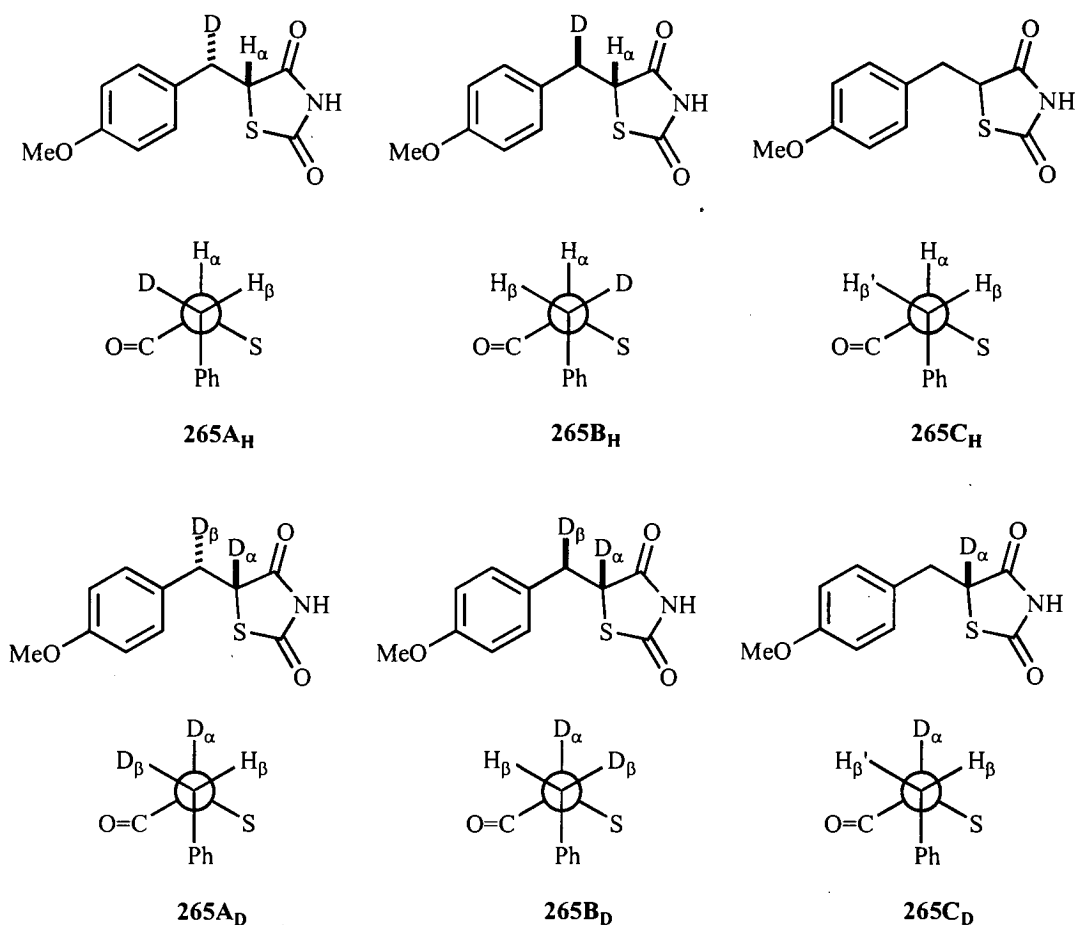


Fig. 5-8 : Proposed Composition of the Product Mixture Based on ¹H nmr.

Peak No.	9	10	11	12	13	14	15	16
Total Relative Integral.	6	6	6	6	11	11	22	15
Relative Integral Arising from 265C_H	6	6	6	6	---	---	---	---
Relative Integral Arising from 265A_H	---	---	---	---	---	11	11	---
Relative Integral Arising from 265C_D	---	---	---	---	11	---	11	---
Total Relative integral Left	0	0	0	0	0	0	0	15
⇒ Relative Integral Arising from 265A_D	---	---	---	---	---	---	---	15

Table 5-1 : Relative Integrals, and Composition of Peaks 9 to 16 from Fig. 5-7d.

Compound	265A _H	265A _D	265C _H	265C _D
Total Integral	22	15	24	22
Relative Ratio	1.47	1.00	1.60	1.47

Table 5-2 : Relative Ratios of 265A_H, 265A_D, 265C_H and 265C_D.

Peak No.	17	18	19	20 and 22	21
Total Relative Integral.	6	6	15	41	11
Relative Integral Arising From 265C _H	6	6	6	6	
Relative Integral Arising From 265B _H	---	---	9	9	
Relative Integral Arising From 265C _D	---	---	---	11	11
Total Relative Integral Left.	0	0	0	15	0
⇒ Relative Integral Arising from 265B _D	---	---	---	15	---

Table 5-3 : Realitive Integrals, and Composition of Peaks 17 to 22 from Fig. 5-7e.

Compound	265B _H	265B _D	265C _H	265C _D
Total Integral	18	15	24	22
Relative Ratio	1.20	1.00	1.60	1.47

Table 5-4 : Relative Ratios of 265B_H, 265B_D, 265C_H and 265C_D.

Peak overlap and manual measurement of integrals leads to significant errors in the calculation of relative ratios. However it could be seen, in terms of the percentage make-up of the mixture, that 265C_H > 265A_H and 265C_D > 265A_D (where 265A_H and 265C_D were similar).

The relative integrals of peaks 17 to 22 arising from H_p at higher field, Fig. 5-7e, are shown in Table 5-3. Due to the extent of overlap the total integral across peaks 20 and 22 is quoted.

265C_H gave rise to peaks 17-20, although 17 and 18 had the same integral 19 and 20 were significantly larger (Table 5-3). If the pattern exhibited in the other H_p region was repeated here then part of 19 and 20 (equal in intensity to 17 and 18) was due to 265C_H. It follows that the remaining integral of peak 19 must have arisen from 265B_H as did a similar portion of peak 20.

This has accounted for the total integrals displayed by peaks 17-19, however it left a fraction of the integral of peak 20/22 unaccounted for. This unaccounted area was greater than the total area of peak 21 and it is proposed that 20 and 21 arose from **265C_D**, if the relative area of 21 is subtracted from the unaccounted portion of 20/22 then it leaves a region of peak 22 which accounts for the obscured singlet arising from **265B_D**.

Again, relative ratios of these four compounds were calculated based on the ratios of the total integral exhibited in this region of the spectrum (Table 5-4). From these ratios it was possible to say that in terms of the percentage make up of the mixture then **265C_H > 265C_D > 265B_H > 265B_D**.

In summary, four conclusions can be drawn from examination of the ¹H nmr. Firstly, there was evidence for the existence of all six isotopomers shown in Fig. 5-8 and from the integrals at H_α the ratio between **265A_H**, **265B_H** and **265C_H** is 1.00:1.00:1.28. The integrals exhibited in the H_β region at lower field (Fig. 5-7d) showed that **265C_H > 265C_D** and **265A_H > 265A_D** (where **265A_H** and **265C_D** are similar) whilst H_β at higher field (Fig. 5-7e) showed that **265C_H > 265C_D > 265B_H > 265B_D**.

Compound	265A_H + 265A_D	265B_H + 265B_D	265A_D + 265B_D + 265C_D
Relative Ratio	1.00	1.00	1.70

Table 5-5 : Relative Ratios as Determined by ²H nmr (Fig. 5-9).

²H nmr showed the relative amount of deuterium incorporated at each of the three positions (Table 5-5 and Fig. 5-9). As the ratio of **265A_H** to **265B_H** was 1:1 (Fig. 5-6b), and the ratio of the totals of **265A** and **265B** was 1:1, it follows that the ratio between **265A_D** and **265B_D** must also be 1:1.

The six isotopomers gave rise to three molecular ion peaks 237, 238 and 239, with an intensity ratio of 1.00:2.46:1.40, as determined by low resolution electron impact

mass spectrometry. The composition of each of these molecular ion signals is shown in Table 5-6.

^1H nmr has shown that the ratio $265\text{A}_\text{H}:265\text{B}_\text{H}:265\text{C}_\text{H}$ is 1.00:1.00:1.28 (or 0.78:0.78:1.00) and therefore the contribution of 265A_H and 265B_H to m/z 238 must have been 1.56, whilst that of 265C_D was 0.90. ^1H and ^2H nmr have also shown that the ratio of $265\text{A}_\text{D}:265\text{B}_\text{D}$ is 1.00:1.00 and therefore each contributed equally to m/z 239, with a value of 0.70 each. This allowed the calculation of the relative ratios of all six isotopomers (Table 5-7). These figures are in accordance with the hypotheses based on both ^1H and ^2H nmr spectra.

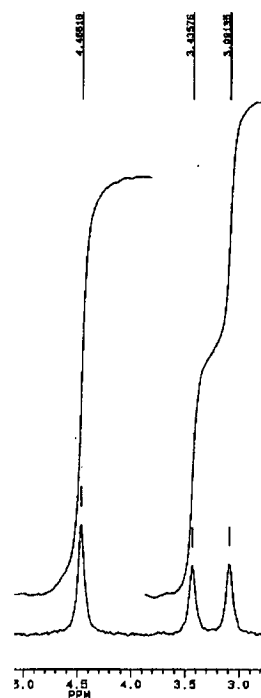


Fig 5-9 : ^2H nmr at 360 MHz of 265.

m/z	Composition	Relative Intensities
237	265C_H	1.00
238	$265\text{C}_\text{D} + 265\text{A}_\text{H} + 265\text{B}_\text{H}$	2.46
239	$265\text{A}_\text{D} + 265\text{B}_\text{D}$	1.40

Table 5-6 : Predicted Composition of m/z Values Along With Relative Intensities.

265A_H	:	265B_H	:	265C_H	:	265A_D	:	265B_D	:	265C_D
0.78	:	0.78	:	1.00	:	0.70	:	0.70	:	0.90

Table 5-7 : Final Calculated Ratios of 265A_H - 265C_H .

Shaking **265**, in CDCl_3 , with sat. aqueous sodium hydrogen carbonate removed D_α to leave 265A_H , 265B_H and 265C_H which greatly simplified the spectroscopic data.

^1H nmr at H_α exhibited the same pattern as before and indicated a ratio between 265A_H , 265B_H and 265C_H of 1.00:1.00:1.29 (compared to the previous 1.00:1.00:1.28). Similarly H_β at lower field showed a ratio between 265A_H and 265C_H of 1.00:1.15 whilst H_β at higher field showed a ratio between 265B_H and 265C_H of 1.00:1.58. This gives an average ratio between 265A_H , 265B_H and 265C_H of 1.00:1.00:1.34.

Electron impact mass spectrometry showed peaks with m/z 237 (265C_H) and 238 (265A_H and 265B_H) in a ratio of 1.30:2.00. This translated to a ratio between 265A_H , 265B_H and 265C_H of 1.00:1.00:1.30.

Both these calculations compared favourably with the previously calculated ratio of 1.00:1.00:1.28 (265A : 265B : 265C).

The level of deuteration at the β position can be calculated as 61 %. Although it is known that the α centre is prone to racemisation the β centre should remain fixed. It was confirmed that the β centre is indeed fixed by performing several simple experiments.

Initially the non-deuterated reduced compound **243**, prepared by catalytic hydrogenation (Chapter 4), was shaken in deuterated buffer and DMSO, in the same proportions as used for the biotransformation to produce **265**, for 7 d. ^1H nmr did not show any evidence of exchange between the H_β positions and the buffer/co-solvent mixture. Similarly the reduced compound **243** was shaken, for 7 d, with *Rhodotorula rubra* CBS 6469 in deuterated buffer/co-solvent. Again, ^1H nmr did not provide any evidence of exchange between the H_β positions and the yeast.

In light of the results of these two experiments it can be said that the 61 % incorporation of deuterium at H_β occurs during the reduction process.

By taking the weight of the cell pellet, resulting from washing and centrifugation, it was possible to lyophilise the pellet to constant weight and hence calculate the amount of water present in the pellet. Furthermore by taking the dry cells and resuspending them in the volume of water that had been removed it was possible to work out the volume taken up by the cells in the final cell suspension. As the total volume of the resultant cell suspension used in the biotransformation was known it was possible to work out the amount of D₂O present as a function of the total amount of 'water' present, this was calculated to be 79 %.

5.3 Attempted Biotransformations to Afford Enantiomerically Enriched Product.

SmithKline Beecham have shown that by reducing the pH from 8.0 to 3.75 it is possible to produce *R*-2, as its hydrochloride monohydrate, in 99.5 % e.e. and 18 % yield.⁷

Due to the racemisation occurring at the α position it is difficult to determine from which enantiotopic face the reductase system adds hydrogen at the β position. It was envisaged that yeast reduction of **215** at low pH, to impart a degree of enantioselectivity at the α position, would simplify the process. Initially it was attempted to reduce **172** at pH 3.75.

The main problem associated with performing such a reaction concerns the measurement of any e.e., as the chiral hplc conditions used by SmithKline Beecham for **2** did not afford any separation of **243**. As the biotransformation is at low pH it is necessary to basify the buffer before the product can be extracted which could lead to racemisation. An ideal situation would thus involve having an on-line method to quantify the e.e. in the reaction mixture before work-up. Several chiral hplc columns were tested with the Chiralpak AD exhibiting excellent separations of **243** and other

reduced standards. However, this system was not robust and reproducing the chromatography proved difficult.

Chiral shift analysis by ^1H nmr, in CDCl_3 , of **243** in the presence of $\text{Eu}(\text{hfc})_3$ afforded separation of the aromatic doublet at lower field and it was envisaged that this could be used to quantify the e.e. of the final product.

Initial attempts involved reproducing the SmithKline Beecham procedure with **172** instead of **6**, however this only produced 22 % conversion after 41 h. 1,4-dioxane was retained as the co-solvent as the use of DMSO would complicate the purification procedure. Reducing the concentration of 1,4-dioxane from 13.6 to 8.5 % v/v, and the concentration of **172** from 2.8 to 2.2 mM, increased conversion to 49 % after 44 h, considerably longer than the 3 h 20 min that the SmithKline Beecham reduction took. The ratio of biomass to substrate was increased with combined cells from 180 and 220 cm^3 of medium being used instead of the cells from 220 cm^3 of medium. These were resuspended in the same volume of 0.1 M citrate buffer and used to reduce **172** (2.2 mM) with 1,4-dioxane (8.5 % v/v) as co-solvent. This resulted in 50 % conversion after 4 h. Using the cells from 2 x 220 cm^3 of growth medium and 1.8 mM **172** resulted in quantitative conversion after 6 h.

Once quantitative conversion had been achieved within a reasonable timescale the cells were removed by centrifugation. However, the supernatant remained slightly cloudy and formed an emulsion on extraction with DCM which was partially avoided by filtration through a 0.45 μm nylon membrane.

Initial attempts to extract the product involved basification to pH 8.0 with 10 % ammonia solution followed by extraction into DCM. The solvent was dried and removed to leave a gummy solid but chiral shift ^1H nmr analysis of this with $\text{Eu}(\text{hfc})_3$ in CDCl_3 showed that the racemate had formed. SmithKline Beecham's reduction of **6** involves dissolving this gummy solid in aqueous hydrochloric acid and allowing the hydrochloride monohydrate of **2** to crystallise overnight at low temperature. It is

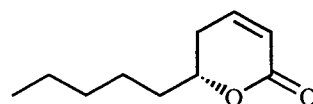
reported that this improves the e.e. from 88 %, at the end of the incubation period, to > 99.5 %. Treatment of the crude product in this manner did produce the salt of **243** in 12 % yield which was insoluble in CDCl_3 .

A racemic sample of **243** hydrochloride monohydrate was prepared and subjected to ^1H nmr chiral shift analysis with $\text{Eu}(\text{hfc})_3$ in $(\text{CD}_3)_2\text{CO}$. Again separation of the aromatic doublet at lower field was achieved. Although examination of the biotransformation product indicated that there may be a slight excess of one enantiomer present, it was not possible to achieve baseline separation of the aromatic peaks to enable quantification of the e.e.

5.4 Conclusions.

Although it is not possible to determine which enantiotopic β face hydrogen is incorporated from it can be seen that when the reaction is performed in D_2O the incorporation of deuterium at the β position is greater than at the α position (61 versus 48 %), even though it is believed that the α centre incorporates deuterium by racemisation.

In the reduction of lactone **112** it has been shown that reduction occurs *via* formal hydride delivery from the β -*re* face.⁵⁷ However when the reduction is carried out in 80



112

% D_2O the incorporation of deuterium at positions 2 and 3 is 70 and 45 % respectively.⁹⁵ These values show that deuterium is incorporated to a lesser extent at the site of hydride addition.

Reduction of **172** in 79 % D_2O was found to incorporate deuterium in 48 and 61 % at the α and β centres respectively which would imply that the polarity of the double bond is reversed with hydride addition occurring at the α position. One possible

mechanism is outlined in Fig. 5-10, which indicates formation of the *R* configuration at the α centre as determined by SmithKline Beecham.⁷

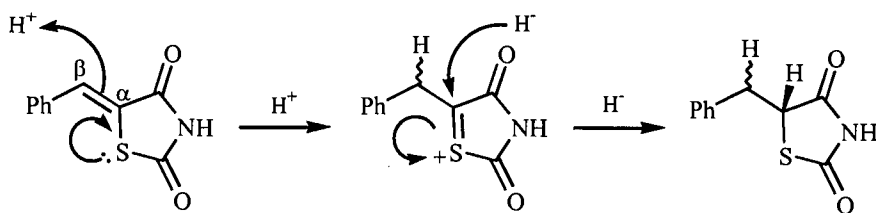


Fig. 5-10 : Possible Mechanism of Reduction By *Rhodotorula rubra* CBS 6469.

Smallridge has shown a similar phenomenon in the baker's yeast reduction of β -nitrostyrenes in petroleum ether and has proposed the mechanism outlined in Fig. 5-11.⁶¹ This mechanism involves a reversible non-stereoselective protonation at the β -carbon followed by stereoselective hydride attack at the α -carbon. However in the reduction by *Rhodotorula rubra* CBS 6469 it is not possible to tell whether or not incorporation of hydrogen at the β position is non-stereoselective.

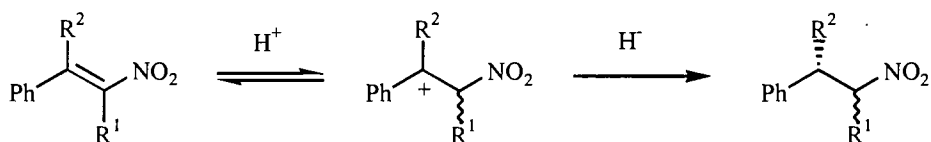


Fig. 5-11 : Mechanism of β -Nitrostyrene Reduction By Baker's Yeast.

Smallridge's proposition for a reversible protonation is based upon two key facts. Firstly that reduction of β -deutero- β -nitrostyrene resulted in an 80 % loss of deuterium during the reduction. By showing that exchange does not occur between the reduced product and the water present, or between reduced product and protons bound to the yeast, he concluded that exchange at the β -centre must be occurring before product formation. Secondly, it was noted that in an incomplete reduction of β -deutero- β -nitrostyrene non-deuterated starting material remained, along with deuterated and non-deuterated reduced compound, indicating that the non-deuterated product resulted from the reduction of the non-deuterated double bond.

Although an incomplete reduction of **215** with *Rhodotorula rubra* CBS 6469 was never attempted there was no loss of deuterium during the reduction indicating that, in this case, incorporation of hydrogen at the β centre is not reversible.

It is also difficult to establish whether or not the proton comes directly from the yeast. Smallridge achieves this by reducing β -deutero- β -nitrostyrene in petroleum ether and D_2O . This resulted in loss of deuterium in both the reduced compound and the recovered starting material from which he concluded that the protonation at the β -centre is not only yeast catalysed but also that the proton is delivered by the yeast rather than coming from the water present. Šunjic has also proposed an enzyme assisted protonation at an emerging chiral centre in the reduction of 2-methyl cinnamaldehyde **26** (Fig. 1-8).

There are two possible explanations for the formation of non-deuterated product when the *Rhodotorula rubra* CBS 6469 reduction is carried out in D_2O . As this reduction is carried out in 79 % D_2O , if the proton is derived from the water present then either a proton or deuteron could be incorporated. Similarly if the proton is delivered by the yeast it is feasible that initially a proton will be transferred which may be replaced by a deuteron in the yeast which is in turn delivered to the substrate. The ability to utilise lyophilised *Rhodotorula rubra* CBS 6469 could have been exploited here.

The necessity for the presence of sulfur could arise from its ability to stabilise an α positive charge. There are two mechanisms for such stabilisation : inductive and resonance stabilisation.

Inductive stabilisation is linked to electronegativity with atoms with a higher electronegativity more readily attracting electrons from the already electron-deficient centre and destabilising the positive charge. On the Pauling scale of electronegativity sulfur has a value of 2.5 whilst nitrogen exhibits an electronegativity of 3.0.

A more powerful effect is that of resonance stabilisation. This involves the donation of electrons into the empty p orbital of the carbocation. Both sulfur and nitrogen possess lone pairs of electrons capable of stabilising the carbocation in this manner. As the heteroatom is adjacent to a carbonyl group the nitrogen lone pair electrons would be delocalised by overlap with this carbonyl group's π orbital, an effect well established in the structure of amides. This would decrease the stabilising effect of the nitrogen lone pair electrons on the α carbocation.

Based upon the observations made, the apparent reversal of polarity of the carbon-carbon double bond compared to Fuganti's lactone reduction, the requirement of the sulfur atom, the possible formation of a racemic centre β to the sulfur atom and the reported reduction at low pH to produce the R enantiomer at the α position, it is feasible to propose a possible mechanism for reduction (Fig. 5-10) which accounts for these observations.

6. Experimental : Synthesis.

6.1 General Experimental.

Melting points were determined using a Gallenkamp Electrothermal Melting Point apparatus and are uncorrected.

Optical rotations were recorded at 589 nm on an AA-1000 polarimeter with a path length of 0.5 dm. Concentrations (c) are given in g/100 cm³ and $[\alpha]_D^T$ values are quoted in 10⁻¹ degcm²g⁻¹.

Tlc was performed on Merck 60F₂₅₄ (0.25 mm) glass backed silica plates. The plates were developed by uv visualisation and by treatment with an aqueous alkaline potassium permanganate solution followed by heating. Column chromatography was performed using Merck silica gel 60H (230-400 mesh). Eluent compositions are quoted as v/v ratios.

Elemental analysis (CHN) was performed on a Perkin Elmer 2400 CHN Elemental Analyser.

Infrared spectra were recorded either as thin films (on sodium chloride plates) or as KBr disks and are denoted by (Film) or (KBr) respectively. Spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR with frequencies being measured in wavenumbers (cm⁻¹).

Nmr spectra were recorded, either on a Bruker AC200, a Bruker AC250, a Bruker WH360 or a Varian Inova 600 spectrometer. Chemical shifts in CDCl₃, [²H₆] DMSO or (CD₃)₂CO are referenced to tetramethylsilane. Spin coupling constants (J) are

measured in Hertz (Hz) and are rounded to the nearest 0.5 Hz whilst chemical shifts (δ) are given in parts per million (ppm). For 1,4-disubstituted benzene rings only the 3J_o coupling constant is quoted. The following abbreviations are used in the assignment of splitting patterns in the nmr spectra : s (singlet); d (doublet); t (triplet); q (quartet); spt (septuplet), dd (doublet of doublets); tspt (triplet of septuplets); tq (triplet of quartets); m (multiplet); br (broad). ^{13}C nmr are broad band decoupled and are assigned as quaternary carbon (qC), methine (CH), methylene (CH_2) and methyl (CH_3) by use of DEPT.

Mass spectrometry was carried out using electron impact (EI) ionisation and a Finnigan 4500 instrument and values are quoted as m/z values.

Hplc was carried out using a Waters 600 Controller/Pump on a Phenomenex Sphereclone 5 μm ODS2 column (250 mm \times 4.6 mm), eluting with 50 mM sodium phosphate buffer (pH 7.0) and acetonitrile at a flow rate of 1.0 $\text{cm}^3\text{min}^{-1}$. Eluent composition varies between reactions and is detailed, with retention times (R_T in min), in Appendix 1. Samples, 10 μl , were injected using a Waters 717+ autosampler with detection at 245 nm using a Waters 486 Tunable Absorbance Detector. Chromatograms were recorded and manipulated using Millenium Chromatography Manager v.2.15 software. All hplc samples were filtered through 0.45 μm nylon syringe filters prior to analysis.

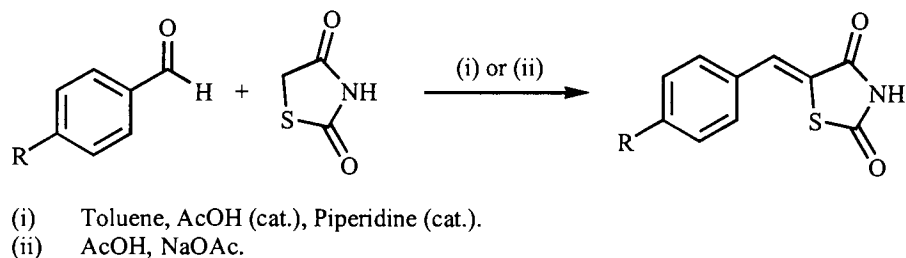
Chiral gc was carried out using a Shimadzu GC-17 on a Supelco Beta-dex fused silica capillary column, 30 m, 0.25 mm ID, 0.25 μm film thickness with helium as carrier gas, 125 $\text{cm}^3\text{min}^{-1}$. The injector had a split ratio of 50:1 and was at 250 $^\circ\text{C}$ whilst the detector was at 300 $^\circ\text{C}$. The column was initially at 150 $^\circ\text{C}$ for 50 min before heating to 250 $^\circ\text{C}$ at 20 $^\circ\text{Cmin}^{-1}$. Chromatograms were monitored and integrated using a Shimadzu C-R6A Chromapac.

Anhydrous reactions were performed in oven dried glassware (overnight at 150 $^\circ\text{C}$) under an argon or nitrogen atmosphere. THF and DCM were dried by distillation

from sodium benzophenone ketyl and calcium hydride respectively. TFA was dried by distillation from trifluoroacetic anhydride and piperidine was dried over potassium hydroxide followed by fractional distillation. Anhydrous diethyl ether and pyridine were purchased from Aldrich. Lipase PS was supplied by Amano Enzyme Europe Ltd.. All other solvents and reagents were purchased from commercial suppliers.

Where literature data is available the citation is denoted by (lit.,^x) after the reported data, except literature melting points and optical rotations which are quoted. For compounds where two regioisomers can exist then the literature is only cited where the appropriate isomer has been previously reported.

6.2 Knoevenagel Condensation Between Thiazolidine-2,4-dione and 4-Substituted Benzaldehydes.



6.2.1 General Procedures.

General Procedure 1 :

Thiazolidine-2,4-dione (47.0 mmol) and 4-substituted benzaldehyde (47.0 mmol) were heated at reflux in toluene (400 cm³), containing piperidine (0.1 cm³) and acetic acid (0.1 cm³), under Dean and Stark conditions. The solution was allowed to cool and the product recovered by filtration and recrystallised from ethanol. The mother liquors were reduced and recrystallised to obtain a second crop of crystals.

General Procedure 2 :

Thiazolidine-2,4-dione (10.0 mmol) and 4-substituted benzaldehyde (10.0 mmol) were heated at reflux in toluene (65 cm³), containing piperidine (0.30 mmol) and acetic acid (0.30 mmol), under Dean and Stark conditions. The solution was allowed to cool and the product recovered by filtration.

General Procedure 3 :

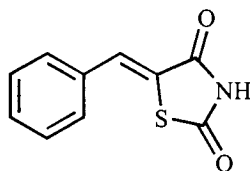
Thiazolidine-2,4-dione (15.0 mmol) and 4-substituted benzaldehyde (15.0 mmol) were heated at reflux in toluene (100 cm³), containing piperidine (0.44 mmol) and

acetic acid (0.44 mmol), under Dean and Stark conditions. The solution was allowed to cool and the product recovered by filtration and recrystallised from ethanol. The mother liquors were reduced and recrystallised to obtain a second crop of crystals.

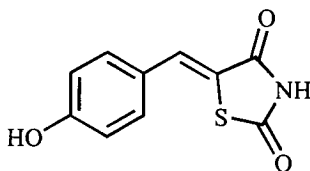
General Procedure 4 :

Thiazolidine-2,4-dione (42.0 mmol) and 4-substituted benzaldehyde (42.0 mmol) were heated at reflux in acetic acid (50 cm³), containing anhydrous sodium acetate (58.0 mmol). The acetic acid was removed by co-evaporation with ethanol and the crude product was washed with water (2 x 50 cm³). The crude product was recrystallised from ethanol and the mother liquors were reduced and recrystallised to obtain a second crop of crystals.

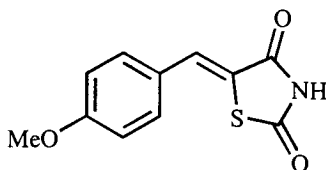
6.2.2 (Z)-5-benzylidenethiazolidine-2,4-dione 170.



General procedure 2, for 27 h, afforded the title compound as a yellow powder on recrystallisation from toluene (1.65 g, 8.0 mmol, 80 %); mp 242-244 °C (From toluene) (lit.,¹⁷³ 230 °C); R_F (EtOAc-hexane, 1:1) 0.46; ν_{\max} (KBr)/cm⁻¹ 3032 (olefinic C-H/Ph-H), 1739 (C=O), 1689 (C=O), 1610 (Ph), 1595 (Ph) and 760 (Ph-H) (lit.,¹⁷³); δ_H (250 MHz; [2H₆] DMSO) 7.51 (5H, m, Ph), 7.77 (1H, s, olefinic H), 12.65 (1H, br s, NH) ppm (lit.,¹⁷³); δ_C (63 MHz; [2H₆] DMSO) 123.66 (qC), 129.46 (CH), 130.17 (CH), 130.57 (CH), 131.95 (CH), 133.17 (qC), 167.47 (qC), 168.05 (qC) ppm; m/z (EI) 205 (M, 38 %) and 134 (M-C(O)NHC(O), 100); (Found: M⁺ (EI), 205.01933. C₁₀H₇NO₂S requires 205.01975).

6.2.3 (Z)-5-(4-hydroxybenzylidene)thiazolidine-2,4-dione 171.

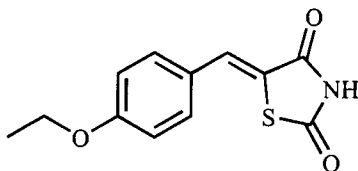
General procedure 2, for 27 h, afforded the title compound as a yellow powder on recrystallisation from ethanol (1.69 g, 7.56 mmol, 76 %); mp 291-293 °C (From toluene) (lit.,¹⁷⁴ >290 °C); R_f (EtOAc-hexane, 1:1) 0.21; ν_{\max} (KBr)/cm⁻¹ 3404 (O-H stretch), 2996 (olefinic C-H/Ph-H), 1726 (C=O), 1677 (C=O), 1592 (Ph), 1573 (Ph), 1510 (Ph) and 826 (Ph-H); δ_H (200 MHz; [²H₆] DMSO) 6.91 (2H, d, J 9.0, Ph), 7.45 (2H, d, J 9.0, Ph), 7.69 (1H, s, olefinic H), 10.36 (1H, br s, OH), 12.46 (1H, br s, NH) ppm; δ_C (50 MHz; [²H₆] DMSO) 102.55 (CH), 116.37 (CH), 119.02 (qC), 123.98 (qC), 132.44 (CH), 159.93 (qC), 167.55 (qC), 168.10 (qC) ppm; m/z (EI) 221 (M, 99 %) and 150 (M-C(O)NHC(O), 100); (Found: M^+ (EI), 221.01477. C₁₀H₇NO₃S requires 221.01466).

6.2.4 (Z)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione 172.

General procedure 1, for 25.5 h, afforded the title compound as a yellow fibrous solid (8.72 g, 37.1 mmol, 75 %); mp 212-213 °C (from EtOH) (lit.,¹⁷³ 210 °C); R_f (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.49; (Found : C, 55.76; H, 3.69; N, 5.80. C₁₁H₉NO₃S requires C, 56.17; H, 3.83; N, 5.96 %); ν_{\max} (KBr)/cm⁻¹ 3017 (olefinic C-H/Ph-H), 2771 (CH₃), 1727 (C=O), 1700 (C=O), 1580 (Ph), 1510 (Ph), 1468 (CH₃), and 823 (Ph-H) (lit.,¹⁷³); δ_H (250 MHz; [²H₆] DMSO) 3.81 (3H, s, OCH₃), 7.07 (2H, d, J 9.0, Ph), 7.53 (2H, d, J 9.0, Ph), 7.72 (1H, s, olefinic H) ppm; δ_C (63MHz; [²H₆] DMSO) 55.57 (CH₃), 115.00 (CH), 120.41 (qC), 125.61 (qC),

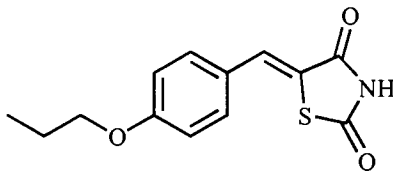
131.94 (CH), 132.19 (CH), 161.11 (qC), 167.58 (qC), 168.09 (qC) ppm; m/z (EI) 235 (M, 31 %) and 164 (M-C(O)NHC(O), 100); (Found M^+ (EI), 235.03067. $C_{11}H_9NO_3S$ requires 235.03032).

6.2.5 (Z)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione 173.



General procedure 3, for 20 h, afforded the title compound as yellow needles (2.41 g, 9.67 mmol, 65 %); mp 189-191 °C (From EtOH); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.69; (Found : C, 57.88; H, 4.42; N, 5.67. $C_{12}H_{11}NO_3S$ requires C, 57.82; H, 4.45; N, 5.62 %); ν_{max} (KBr)/ cm^{-1} 2983 (CH_2/CH_3), 1739 (C=O), 1686 (C=O), 1593 (Ph), 1568 (Ph), 1510 (Ph), 1478 (CH_2/CH_3), 1392 (CH_3), 828 (Ph-H) and 719 (CH_2); δ_H (250 MHz; [2H_6] DMSO) 1.32 (3H, t, J 7.0, OCH_2CH_3), 4.05 (2H, q, J 7.0, OCH_2CH_3), 7.02 (2H, d, J 9.0, Ph), 7.47 (2H, d, J 9.0, Ph), 7.68 (1H, s, olefinic H), 12.48 (1H, br s, NH) ppm; δ_C (63MHz; [2H_6] DMSO) 14.59 (CH_3), 63.60 (CH_2), 115.31 (CH), 120.17 (qC), 125.41 (qC), 131.98 (CH), 132.19 (CH), 160.40 (qC), 167.50 (qC), 168.03 (qC) ppm; m/z (EI) 249 (M, 27 %) and 178 (M-C(O)NHC(O), 100); (Found : M^+ (EI), 249.04600 ($C_{12}H_{11}NO_3S$ requires 249.04597).

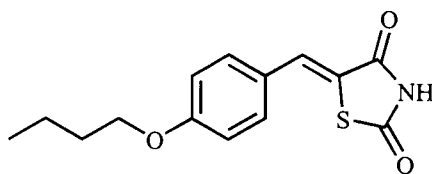
6.2.6 (Z)-5-(4-*n*-propoxybenzylidene)thiazolidine-2,4-dione 174.



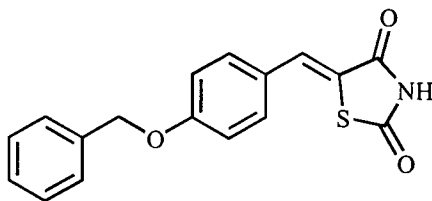
General procedure 3, for 18 h, afforded the title compound as a yellow powder (2.81 g, 10.7 mmol, 71 %); mp 189-190 °C (From EtOH); R_F (EtOAc-petroleum ether (bp

40-65 °C), 1:1) 0.66; (Found : C, 59.29; H, 4.96; N, 5.30. $C_{13}H_{13}NO_3S$ requires C, 59.30; H, 4.98; N, 5.32 %); ν_{\max} (KBr)/ cm^{-1} 2964 (CH_2/CH_3), 1736 (C=O), 1687 (C=O), 1591 (Ph), 1568 (Ph), 1513 (Ph), 1474 (CH_2/CH_3), 828 (Ph-H) and 729 (CH_2); δ_H (250 MHz; [2H_6] DMSO) 0.95 (3H, t, J 7.5, $OCH_2CH_2CH_3$), 1.71 (2H, dd, J 7.0 7.5, $OCH_2CH_2CH_3$), 3.94 (2H, t, J 7.0, $OCH_2CH_2CH_3$), 7.01 (2H, d, J 9.0, Ph), 7.47 (2H, d, J 9.0, Ph), 7.68 (1H, s, olefinic H), 12.47 (1H, br s, NH) ppm; δ_C (63MHz; [2H_6] DMSO) 10.38 (CH_3), 22.03 (CH_2), 69.36 (CH_2), 115.31 (CH), 120.16 (qC), 125.39 (qC), 131.96 (CH), 132.16 (CH), 160.53 (qC), 167.49 (qC), 168.00 (qC) ppm; m/z (EI) 263 (M, 24 %) and 192 (M-C(O)NHC(O), 50); (Found M^+ (EI), 263.06124. $C_{13}H_{13}NO_3S$ requires 263.06162).

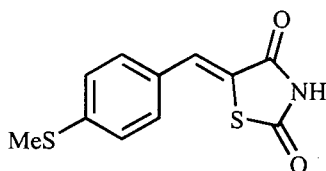
6.2.7 (Z)-5-(4-*n*-butoxybenzylidene)thiazolidine-2,4-dione **175**.



General procedure 3, for 22.5 h, afforded the title compound as a yellow powder (3.49 g, 12.6 mmol, 84 %); mp 171-172 °C (From EtOH); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.67; (Found : C, 60.86; H, 5.55; N, 4.99. $C_{14}H_{15}NO_3S$ requires C, 60.63; H, 5.46; N, 5.05 %); ν_{\max} (KBr)/ cm^{-1} 2962 (CH_2/CH_3), 1741 (C=O), 1693 (C=O), 1588 (Ph), 1511 (Ph), 1469 (CH_2/CH_3), 1389 (CH_3) and 830 (Ph-H); δ_H (250 MHz; [2H_6] DMSO) 0.91 (3H, t, J 7.0, $OCH_2CH_2CH_2CH_3$), 1.43 (2H, tq, J 7.0, $OCH_2CH_2CH_2CH_3$), 1.67 (2H, tt, J 6.5 7.0, $OCH_2CH_2CH_2CH_3$), 4.00 (2H, t, J 6.5, $OCH_2CH_2CH_2CH_3$), 7.04 (2H, d, J 9.0, Ph), 7.50 (2H, d, J 9.0, Ph), 7.71 (1H, s, olefinic H), 12.49 (1H, br s, NH) ppm; δ_C (63MHz; [2H_6] DMSO) 13.74 (CH_3), 18.78 (CH_2), 30.69 (CH_2), 67.62 (CH_2), 115.36 (CH), 120.17 (qC), 125.40 (qC), 131.98 (CH), 132.17 (CH), 160.56 (qC), 167.49 (qC), 168.01 (qC) ppm; m/z (EI) 277 (M, 25 %) and 206 (M-C(O)NHC(O), 37); (Found M^+ (EI), 277.07800. $C_{14}H_{15}NO_3S$ requires 277.07727).

6.2.8 (Z)-5-(4-benzyloxybenzylidene)thiazolidine-2,4-dione 178.

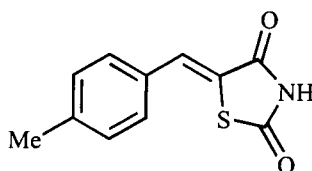
General procedure 3, for 24 h, afforded the title compound as a yellow powder (3.79 g, 12.2 mmol, 82 %); mp 218-220 °C (From EtOH); R_F (EtOAc-hexane, 1:1) 0.44; (Found : C, 65.78; H, 4.35; N, 4.49. $C_{17}H_{13}NO_3S$ requires C, 65.58; H, 4.21; N, 4.50 %); ν_{\max} (KBr)/ cm^{-1} 3031 (olefinic C-H/Ph-H), 2870 (CH_2), 1744 (C=O), 1693 (C=O), 1600 (Ph), 1565 (Ph), 1508 (Ph), 1456 (CH_2), 824 (Ph-H) and 724 (Ph-H); δ_H (250 MHz; $[\text{}^2\text{H}_6]$ DMSO) 5.18 (2H, s, OCH_2Ph), 7.16 (2H, d, J 9.0, Ph), 7.40 (5H, m, Ph), 7.55 (2H, d, J 9.0, Ph), 7.74 (1H, s, olefinic H), 12.52 (1H, br s, NH) ppm; δ_C (63MHz; $[\text{}^2\text{H}_6]$ DMSO) 69.59 (CH_2), 115.79 (CH), 120.49 (qC), 125.77 (qC), 127.90 (CH), 128.11 (CH), 128.58 (CH), 131.89 (CH), 132.16 (CH), 136.57 (qC), 160.16 (qC), 167.50 (qC), 168.03 (qC) ppm; m/z (EI) 311 (M, 17 %) and 149 (M- $\text{C}(\text{O})\text{NHC}(\text{O})\text{-CH}_2\text{Ph}$, 9); (Found M^+ (EI), 311.06120. $C_{17}H_{13}NO_3S$ requires 311.06162).

6.2.9 (Z)-5-(4-methylsulfanylbenzylidene)thiazolidine-2,4-dione 179.

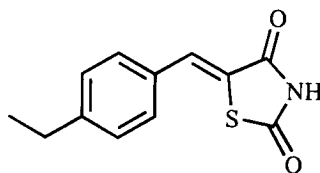
General procedure 3, for 24 h, afforded the title compound as a yellow solid (2.72 g, 10.8 mmol, 72 %); mp 206-208 °C (From EtOH); R_F (EtOAc-hexane, 1:1) 0.50; ν_{\max} (KBr)/ cm^{-1} 2998 (olefinic C-H/Ph-H), 1737 (C=O), 1685 (C=O), 1605 (Ph), 1585 (Ph), 1430 (CH_3) and 814 (Ph-H); δ_H (250 MHz; $[\text{}^2\text{H}_6]$ DMSO) 2.48 (3H, s, SCH_3),

7.33 (2H, d, J 8.5, Ph), 7.46 (2H, d, J 8.5, Ph), 7.69 (1H, s, olefinic H), 12.56 (1H, br s, NH) ppm; δ_c (63MHz; [2H_6] DMSO) 14.16 (CH₃), 122.03 (qC), 125.78 (CH), 129.20 (qC), 130.58 (CH), 131.58 (CH), 142.47 (qC), 167.49 (qC), 167.93 (qC) ppm; m/z (EI) 251 (M, 69 %) and 180 (M-C(O)NHC(O), 100); (Found M^+ (EI), 251.00738. C₁₁H₉NO₂S₂ requires 251.00747).

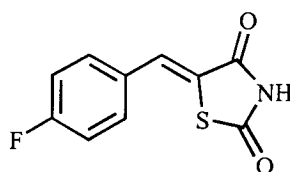
6.2.10 (Z)-5-(4-methylbenzylidene)thiazolidine-2,4-dione 180.



General procedure 3, for 24 h, afforded the title compound as a yellow powder (1.20 g, 5.48 mmol, 37 %); mp 222-224 °C (From EtOH); R_f (EtOAc-hexane, 1:1) 0.44; ν_{max} (KBr)/cm⁻¹ 3050 (olefinic C-H/Ph-H), 1737 (C=O), 1686 (C=O), 1600 (Ph), 1510 (Ph), 1340 (CH₃) and 828 (Ph-H); δ_H (200 MHz; [2H_6] DMSO) 2.35 (3H, s, CH₃), 7.34 (2H, d, J 8.5, Ph), 7.48 (2H, d, J 8.5, Ph), 7.74 (1H, s, olefinic H) ppm; δ_c (50MHz; [2H_6] DMSO) 21.19 (CH₃), 122.49 (qC), 130.06 (CH), 130.18 (CH), 130.39 (qC), 131.94 (CH), 140.82 (qC), 167.59 (qC), 168.09 (qC) ppm; m/z (EI) 219 (M, 56 %) and 148 (M-C(O)NHC(O), 100); (Found M^+ (EI), 219.03536. C₁₁H₉NO₂S requires 219.03540).

6.2.11 (Z)-5-(4-ethylbenzylidene)thiazolidine-2,4-dione 181.

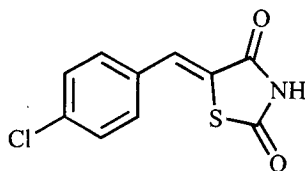
General procedure 3, for 24 h, afforded the title compound as a yellow powder (2.73 g, 11.7 mmol, 78 %); mp 183-185 °C (From EtOH); R_F (EtOAc-hexane, 1:1) 0.53; (Found : C, 61.52; H, 4.91; N, 6.10. $C_{12}H_{11}NO_2S$ requires C, 61.79; H, 4.76; N, 6.01 %); ν_{\max} (KBr)/ cm^{-1} 3028 (olefinic C-H/Ph-H), 2966 (CH_2/CH_3), 1737 (C=O), 1688 (C=O), 1600 (Ph), 1508 (Ph), 1434 (CH_2/CH_3), 1380 (CH_3) and 833 (Ph-H); δ_H (250 MHz; $[\text{}^2\text{H}_6]$ DMSO) 1.15 (3H, t, J 7.5, CH_2CH_3), 2.60 (2H, d, J 7.5, CH_2CH_3), 7.31 (2H, d, J 9.0, Ph), 7.44 (2H, d, J 9.0, Ph), 7.70 (1H, s, olefinic H) ppm; δ_C (63MHz; $[\text{}^2\text{H}_6]$ DMSO) 15.33 (CH_3), 28.33 (CH_2), 122.49 (qC), 128.91 (CH), 130.37 (CH), 130.68 (CH), 132.10 (qC), 146.99 (qC), 167.58 (qC), 168.14 (qC) ppm; m/z (EI) 233 (M, 52 %) and 162 (M-C(O)NHC(O), 100); (Found M^+ (EI), 233.05059. $C_{12}H_{11}NO_2S$ requires 233.05105).

6.2.12 (Z)-5-(4-fluorobenzylidene)thiazolidine-2,4-dione 185.

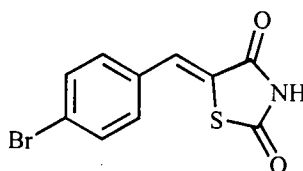
General procedure 3, for 24 h, afforded the title compound as a yellow powder (2.17 g, 9.73 mmol, 65 %); mp 215-216 °C (From EtOH); R_F (EtOAc-petroleum ether (bp 40-65 °C), 2:1) 0.59; (Found : C, 53.81; H, 2.70; N, 6.36. $C_{10}H_6NO_2SF$ requires C, 53.81; H, 2.71; N, 6.28 %); ν_{\max} (KBr)/ cm^{-1} 3043 (olefinic C-H/Ph-H), 1728 (C=O), 1700 (C=O), 1595 (Ph), 1585 (Ph), 1506 (Ph), 1250 (C-F), and 832 (Ph-H); δ_H (250 MHz; $[\text{}^2\text{H}_6]$ DMSO) 7.36 (1H, d, J 9.0, Ph), 7.40 (1H, d, J 9.0, Ph), 7.66 (1H, d, J

9.0, Ph), 7.68 (1H, d, J 9.0, Ph), 7.80 (1H, s, olefinic H) ppm; δ_c (63MHz; [2H_6] DMSO) 121.40 (CH), 121.74 (CH), 128.31 (qC), 134.81 (qC), 135.78 (CH), 137.47 (CH), 137.61 (CH), 165.93 (qC), 172.35 (qC), 172.83 (qC) ppm; m/z (EI) 223 (M, 21 %) and 152 (M-C(O)NHC(O), 100); (Found M^+ (EI), 223.01095. $C_{10}H_6NO_2SF$ requires 223.01033).

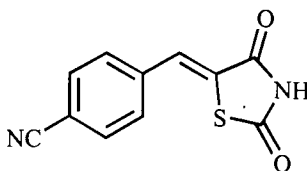
6.2.13 (Z)-5-(4-chlorobenzylidene)thiazolidine-2,4-dione **186**.



General procedure 1, for 47 h, afforded the title compound as a yellow solid (4.35 g, 18.2 mmol, 40 %); mp 228-229 °C (From EtOH); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.52; (Found : C, 49.87; H, 2.33; N, 5.60. $C_{10}H_6NO_2SCl$ requires C, 50.10; H, 2.51; N, 5.85 %); ν_{max} (KBr)/ cm^{-1} 3050 (olefinic C-H/Ph-H), 1757 (C=O), 1693 (C=O), 1607 (Ph), 1552 (Ph), 816 (Ph-H) and 741 (C-Cl); δ_H (250 MHz; [2H_6] DMSO), 7.55 (4H, s, Ph), 7.74 (1H, s, olefinic H) ppm; δ_c (63MHz; [2H_6] DMSO) 124.38 (qC), 129.42 (CH), 130.51 (CH), 131.67 (CH), 132.00 (qC), 135.10 (qC), 167.27 (qC), 167.67 (qC) ppm; m/z (EI) 239 (M(^{35}Cl), 35 %) and 168 (M-C(O)NHC(O), 100); (Found M^+ (EI), 238.98182. $C_{10}H_6NO_2S^{35}Cl$ requires 238.98078).

6.2.14 (Z)-5-(4-bromobenzylidene)thiazolidine-2,4-dione 187.

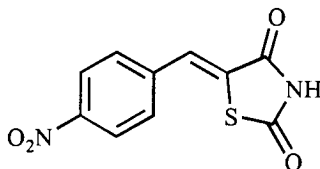
General procedure 3, for 24 h, afforded the title compound as a yellow solid (1.76 g, 6.20 mmol, 41 %); mp 231-233 °C (From EtOH); R_F (EtOAc-hexane, 1:1) 0.47; (Found : C, 42.25; H, 2.38; N, 4.84. $C_{10}H_6NO_2SBr$ requires C, 42.41; H, 2.14; N, 4.95 %); ν_{max} (KBr)/ cm^{-1} 3051 (olefinic C-H/Ph-H), 1748 (C=O), 1719 (C=O), 1610 (Ph), 1578 (Ph), 816 (Ph-H) and 696 (C-Br); δ_H (250 MHz; [2H_6] DMSO), 7.53 (2H, d, J 8.5, Ph), 7.72 (2H, d, J 8.5, Ph), 7.76 (1H, s, olefinic H) ppm; δ_C (63MHz; [2H_6] DMSO) 124.03 (qC), 124.53 (qC), 130.63 (CH), 131.90 (CH), 132.43 (CH), 167.34 (qC), 167.73 (qC) ppm; m/z (EI) 283 ($M(^{79}Br)$, 98 %) and 212 ($M-C(O)NHC(O)$, 69); (Found M^+ (EI), 282.93154 $C_{10}H_6NO_2S^{79}Br$ requires 282.93026).

6.2.15 (Z)-5-(4-cyanobenzylidene)thiazolidine-2,4-dione 189.

General procedure 4, for 3 h, afforded the title compound as a yellow powder (7.48 g, 32.5 mmol, 77 %); mp 315 °C dec. (From EtOH); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.48; ν_{max} (KBr)/ cm^{-1} 3003 (olefinic C-H/Ph-H), 2229 ($C\equiv N$), 1717 (C=O), 1696 (C=O), 1609 (Ph), 1572 (Ph), 1504 (Ph) and 834 (Ph-H); δ_H (250 MHz; [2H_6] DMSO) 7.59 (1H, s, olefinic H), 7.72 (2H, d, J 8.5, Ph), 7.92 (2H, d, J 8.5, Ph) ppm; δ_C (63MHz; [2H_6] DMSO) 110.82 (qC), 118.80 (qC), 125.02 (CH), 130.04 (CH), 132.90 (CH), 133.98 (qC), 139.25 (qC), 171.21 (qC), 174.94 (qC) ppm; m/z

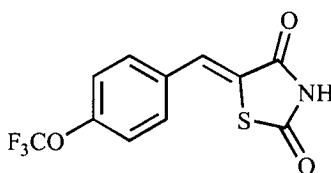
(EI) 230 (M, 14 %) and 159 (M-C(O)NHC(O), 100); (Found M⁺ (EI), 230.01478. C₁₁H₆N₂O₂S requires 230.01450).

6.2.16 (Z)-5-(4-nitrobenzylidene)thiazolidine-2,4-dione **190**.



General procedure 4, for 21 h, afforded the title compound as a light brown powder (7.78 g, 31.1 mmol, 78 %); mp 265-266 °C (From EtOH); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.64; (Found : C, 47.99; H, 2.55; N, 11.10. C₁₀H₆N₂O₄S requires C, 48.00; H, 2.40; N, 11.20 %); ν_{\max} (KBr)/cm⁻¹ 1752 (C=O), 1675 (C=O), 1607 (Ph), 1593 (Ph), 1532 (NO₂), 1348 (NO₂) and 847 (Ph-H); δ_{H} (250 MHz; [²H₆] DMSO) 7.75 (2H, d, *J* 9.0, Ph), 7.81 (1H, s, olefinic *H*), 8.27 (2H, d, *J* 9.0, Ph) ppm; δ_{C} (63MHz; [²H₆] DMSO) 124.01 (CH), 128.00 (qC), 129.02 (CH), 130.71 (CH), 139.36 (qC), 147.37 (qC), 166.89 (qC), 167.03 (qC) ppm; *m/z* (EI) 250 (M, 21 %) and 179 (M-C(O)NHC(O), 100); (Found M⁺ (EI), 250.00483. C₁₀H₆N₂O₄S requires 250.00483).

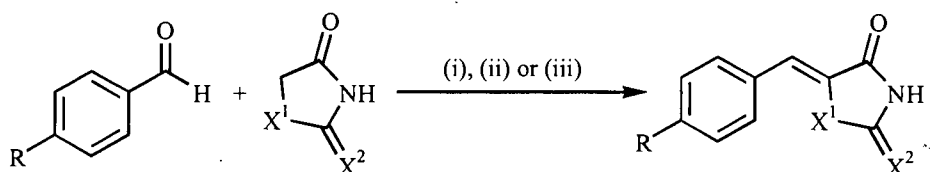
6.2.17 (Z)-5-(4-trifluoromethoxybenzylidene)thiazolidine-2,4-dione **191**.



General procedure 3, for 30 h, afforded the title compound as a yellow powder (2.67 g, 9.24 mmol, 62 %); mp 146-148 °C (From EtOH); R_F (EtOAc-hexane, 1:1) 0.56; (Found : C, 45.39; H, 1.75; N, 5.00. C₁₁H₆NO₃SF₃ requires C, 45.67; H, 2.09; N, 4.85

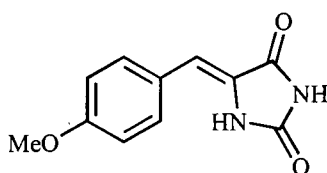
%); ν_{\max} (KBr)/ cm^{-1} 3046 (olefinic C-H/Ph-H), 1755 (C=O), 1722 (C=O), 1611 (Ph), 1584 (Ph), 1507 (Ph), 1282 (C-F) and 826 (Ph-H); δ_{H} (250 MHz; $[\text{}^2\text{H}_6]$ DMSO) 7.45 (2H, d, J 8.5, Ph), 7.67 (2H, d, J 8.5, Ph), 7.77 (1H, s, olefinic H) ppm; δ_{C} (63MHz; $[\text{}^2\text{H}_6]$ DMSO) 118.03 (qC), 121.55 (CH), 124.81 (qC), 130.22 (CH), 132.05 (CH), 132.32 (qC), 149.22 (qC), 167.27 (qC), 167.70 (qC) ppm; m/z (EI) 289 (M, 33 %) and 218 (M-C(O)NHC(O), 100); (Found M^+ (EI), 289.00147. $\text{C}_{11}\text{H}_6\text{NO}_3\text{SF}_3$ requires 289.00205).

6.3 Knoevenagel Condensation Between 4-Substituted Benzaldehydes and Other Five-Membered Heterocycles.



- (i) Toluene, AcOH (cat.), Piperidine (cat.).
- (ii) Piperidine, Δ .
- (iii) NH_4Cl (aq.), NH_3 (aq.).

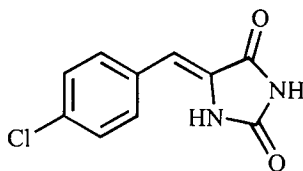
6.3.1 (Z)-5-(4-methoxybenzylidene)imidazolidine-2,4-dione **192**.



4-Methoxybenzaldehyde (1.39 g, 10.2 mmol) and imidazolidine-2,4-dione (1.01 g, 10.1 mmol) in anhydrous piperidine (2 cm^3) were heated to 130 $^\circ\text{C}$, under argon, for 75 min. The heating was removed and hot water (60 $^\circ\text{C}$, 40 cm^3) added before stirring for 2.5 h, whilst being allowed to cool to ambient temperature. The resulting green solution was filtered and the filtrate acidified with 10 M hydrochloric acid (5 cm^3). The solution was allowed to stand overnight. The precipitate was recovered by

filtration, dried under vacuum and recrystallised from acetic acid. Traces of acetic acid were removed by co-evaporation with ethanol to afford the title compound as a yellow powder (811 mg, 3.72 mmol, 37 %); mp 249-250 °C (From AcOH) (lit.,¹⁷⁵ 247 °C); R_f (EtOAc-hexane, 1:1) 0.23; ν_{\max} (KBr)/cm⁻¹ 3040 (olefinic C-H/Ph-H), 1757 (C=O), 1712 (C=O), 1654 (C=C), 1601 (Ph), 1517 (Ph), 1438 (CH₃), 1371 (CH₃) and 825 (Ph-H) (lit.,^{175,176}); δ_H (250 MHz; [²H₆] DMSO) 3.79 (3H, s, OCH₃), 6.39 (1H, s, olefinic *H*), 6.96 (2H, d, *J* 9.0, Ph), 7.59 (2H, d, *J* 9.0, Ph), 10.47 (1H, br s, NH), 11.17 (1H, br s, NH) ppm (lit.,¹⁷⁵⁻¹⁷⁷); δ_C (63MHz; [²H₆] DMSO) 55.38 (CH₃), 108.80 (CH), 114.41 (CH), 125.55 (qC), 126.17 (qC), 131.22 (CH), 155.78 (qC), 159.55 (qC), 165.74 (qC) ppm (lit.,^{176,177}); *m/z* (EI) 218 (M, 90 %) and 147 (M-C(O)NHC(O), 100); (Found M^+ (EI), 218.06933. C₁₁H₁₀N₂O₃ requires 218.06914).

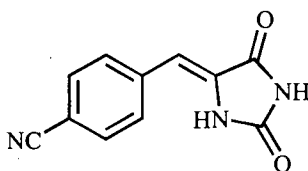
6.3.2 (Z)-5-(4-chlorobenzylidene)imidazolidine-2,4-dione 193.



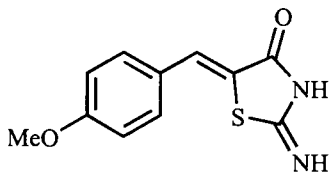
4-Chlorobenzaldehyde (1.41 g, 10.5 mmol) and imidazolidine-2,4-dione (1.05 g, 10.5 mmol) in anhydrous piperidine (2 cm³) were heated to 130 °C, under argon, for 100 min. The heating was removed and hot water (60 °C, 40 cm³) added before stirring for 1.5 h, whilst being allowed to cool to ambient temperature. The solution was acidified with 10 M hydrochloric acid (5 cm³). This was stirred for a further 30 min before the precipitate was recovered by filtration, dried under vacuum and recrystallised from acetic acid. Traces of acetic acid were removed by co-evaporation with ethanol to afford the title compound as a yellow powder (571 mg, 2.56 mmol, 26 %); mp 293-294 °C (From AcOH) (lit.,¹⁷⁶ 295.5-296 °C); R_f (EtOAc-hexane, 1:1) 0.41; ν_{\max} (KBr)/cm⁻¹ 3074 (olefinic C-H/Ph-H), 1798 (C=O), 1737 (C=O), 1666 (C=C), 1588 (Ph), 826 (Ph-H) and 654 (C-Cl) (lit.,¹⁷⁶); δ_H (250 MHz; [²H₆] DMSO)

6.40 (1H, s, olefinic *H*), 7.44 (2H, d, *J* 8.5, Ph), 7.64 (2H, d, *J* 8.5, Ph), 10.62 (1H, br s, *NH*), 11.29 (1H, br s, *NH*) (lit.,^{176,177}) ppm; δ_c (63MHz; [²H₆] DMSO) 106.91 (CH), 125.58 (qC), 128.84 (CH), 131.13 (CH), 132.05 (qC), 132.89 (qC), 155.80 (qC), 165.55 (qC) ppm (lit.,^{176,177}); *m/z* (EI) 222 (M(³⁵Cl), 99 %) and 151 (M-C(O)NHC(O), 100); (Found *M*⁺ (EI), 222.02015. C₁₀H₇N₂O₂³⁵Cl requires 222.01961).

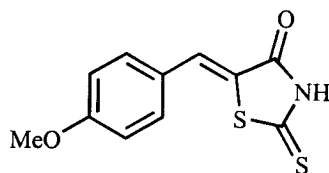
6.3.3 (Z)-5-(4-cyanobenzylidene)imidazolidine-2,4-dione **194**.



4-Cyanobenzaldehyde (1.32 g, 10.1 mmol) and imidazolidine-2,4-dione (1.06 g, 10.6 mmol) in anhydrous piperidine (2 cm³) were heated to 130 °C, under argon, for 30 min. The heating was removed and hot water (60 °C, 40 cm³) added before acidification with 10 M hydrochloric acid (5 cm³). This was stirred for 1 h before the precipitate was recovered by filtration, dried under vacuum and recrystallised from acetic acid. Traces of acetic acid were removed by co-evaporation with ethanol to afford the title compound as a yellow solid (542 mg, 2.55 mmol, 26 %); mp 319 °C dec. (From AcOH) (lit.,¹⁷⁷ 322 °C dec. (From AcOH)); *R_F* (EtOAc-hexane, 1:1) 0.27; ν_{\max} (KBr)/cm⁻¹ 3048 (olefinic C-H/Ph-H), 2222 (C≡N), 1798 (C=O), 1737 (C=O), 1666 (C=C), 1588 (Ph) and 837 (Ph-H); δ_H (250 MHz; [²H₆] DMSO) 6.44 (1H, s, olefinic *H*), 7.78 (2H, d, *J* 8.5, Ph), 7.84 (2H, d, *J* 8.5, Ph), 11.15 (2H, br s, *NH*) ppm (lit.,¹⁷⁷); δ_c (63MHz; [²H₆] DMSO) 105.90 (CH), 110.00 (qC), 118.91 (qC), 129.94 (CH), 130.39 (qC), 132.56 (CH), 137.98 (qC), 155.85 (qC), 165.43 (qC) ppm (lit.,¹⁷⁷); *m/z* (EI) 213 (M, 51 %) and 142 (M-C(O)NHC(O), 100); (Found *M*⁺ (EI), 213.05325. C₁₁H₇N₃O₂ requires 213.05383).

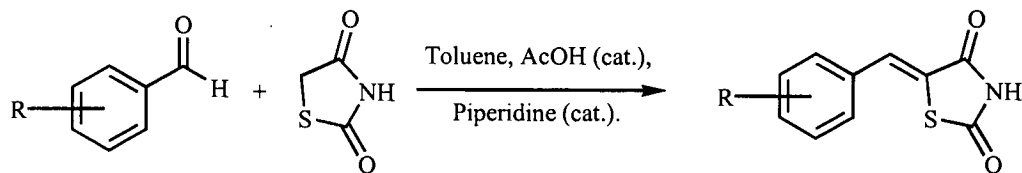
6.3.4 (Z)-5-(4-methoxybenzylidene)thiazolidine-2-imine-4-one 195.

4-Methoxyacetophenone (2.04 g, 15.0 mmol) and thiazolidine-2-imine-4-one (1.74 g, 15.0 mmol) were heated at reflux in toluene (30 cm³), containing piperidine (38 mg, 0.45 mmol) and acetic acid (27 mg, 0.45 mmol), under Dean and Stark conditions, for 21 h. The reaction mixture was allowed to cool and the crude product recovered by filtration and recrystallised from acetic acid. Traces of acetic acid were removed by stirring the product with water (150 cm³) for ca. 1 h. This was repeated three times, removing the water by filtration each time, before washing with diethyl ether (2 x 50 cm³) to afford the title compound as a light orange/brown powder (1.06 g, 4.50 mmol, 30 %); mp 290-291 °C dec.; ν_{\max} (KBr)/cm⁻¹ 3191 (=N-H), 3002 (olefinic C-H/Ph-H), 2834 (CH₃), 1673 (C=O), 1600 (Ph), 1505 (Ph), 1420 (CH₃), 1373 (CH₃) and 820 (Ph-H); δ_{H} (250 MHz; [²H₆] DMSO) 3.80 (3H, s, OCH₃), 7.07 (2H, d, *J* 9.0, Ph), 7.52 (2H, d, *J* 9.0, Ph), 7.55 (1H, s, olefinic *H*), 9.15 (1H, br s, NH), 9.38 (1H, br s, NH) ppm; δ_{C} (63MHz; [²H₆] DMSO) 55.52 (CH₃), 114.89 (CH), 126.61 (qC), 129.19 (CH), 131.40 (CH), 160.42 (qC), 175.59 (qC), 180.77 (qC) ppm; *m/z* (EI) 234 (M, 56 %), 233 (M-H, 63) and 164 (M-C(NH)NHC(O), 81); (Found M⁺ (EI), 234.04723. C₁₁H₁₀N₂O₂S requires 234.04630).

6.3.5 (Z)-5-(4-methoxybenzylidene)thiazolidine-2-thione-4-one **196**.¹⁴¹

Thiazolidine-2-thione-4-one (2.29 g, 17.2 mmol) was added to a suspension of 4-methoxybenzaldehyde (2.33 g, 17.1 mmol) in 32 % aqueous ammonia (1.5 cm³), followed by a solution of ammonium chloride (1.50 g, 28.1 mmol) in hot water (3 cm³). The mixture was stirred for approximately 5 min, until it had solidified, before water (100 cm³) was added and the suspension filtered. The resulting solid was recrystallised from ethanol, and a second crop of crystals obtained from the mother liquors, to afford the title compound as orange needles (2.55 g, 10.2 mmol, 59 %); mp 250-251 °C (From EtOH); *R*_F (EtOAc-hexane, 1:1) 0.62; ν_{\max} (KBr)/cm⁻¹ 3006 (olefinic C-H/Ph-H), 2838 (CH₃), 1686 (C=O), 1584 (Ph), 1565 (Ph), 1510 (Ph), 1445 (CH₃), 1200 (C=S) and 824 (Ph-H); δ_{H} (250 MHz; [²H₆] DMSO) 3.82 (3H, s, OCH₃), 7.07 (2H, d, *J* 9.0, Ph), 7.52 (2H, d, *J* 9.0, Ph), 7.56 (1H, s, olefinic *H*) ppm; δ_{C} (63MHz; [²H₆] DMSO) 55.63 (CH₃), 115.13 (CH), 122.28 (qC), 125.54 (qC), 131.94 (CH), 132.74 (CH), 161.41 (qC), 169.46 (qC), 195.51 (qC) ppm; *m/z* (EI) 251 (M, 29 %) and 164 (M-C(S)NHC(O), 48); (Found *M*⁺ (EI), 251.00774. C₁₁H₉NO₂S₂ requires 251.00747).

6.4 Knoevenagel Condensation Between Thiazolidine-2,4-dione and 2-, 3-, Di- or Tri-substituted Benzaldehydes.



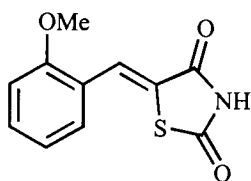
6.4.1 General Procedure.

General Procedure 1 :

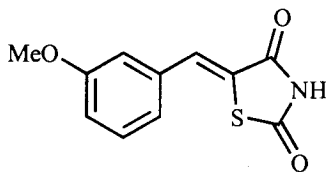
Thiazolidine-2,4-dione (15.0 mmol) and the appropriate benzaldehyde (15.0 mmol) were heated at reflux in toluene (40 cm³), containing piperidine (0.45 mmol) and acetic acid (0.45 mmol), under Dean and Stark conditions. The solution was allowed to cool and the product recovered by filtration.

General Procedure 2 :

Thiazolidine-2,4-dione (6.00 mmol) and the appropriate benzaldehyde (6.00 mmol) were heated at reflux in toluene (40 cm³), containing piperidine (0.18 mmol) and acetic acid (0.18 mmol), under Dean and Stark conditions. The solution was allowed to cool and the product recovered by filtration and recrystallised from ethanol. The mother liquors were reduced and recrystallised to obtain a second crop of crystals.

6.4.2 (Z)-5-(2-methoxybenzylidene)thiazolidine-2,4-dione 197.

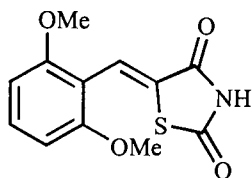
General procedure 1, for 24 h, afforded the title compound as a yellow powder on recrystallisation from ethanol (2.72 g, 11.6 mmol, 77 %); mp 238-239 °C (From EtOH); R_F (EtOAc-hexane, 1:1) 0.38; ν_{\max} (KBr)/ cm^{-1} 3012 (olefinic C-H/Ph-H), 2841 (CH_3), 2770 (CH_3), 1740 ($\text{C}=\text{O}$), 1695 ($\text{C}=\text{O}$), 1586 (Ph), 1461 (CH_3) and 755 (Ph-H); δ_H (250 MHz; [$^2\text{H}_6$] DMSO) 3.85 (3H, s, OCH_3), 7.06 (2H, m, Ph), 7.40 (2H, m, Ph), 7.94 (1H, s, olefinic H), 12.53 (1H, br s, NH) ppm; δ_C (63MHz; [$^2\text{H}_6$] DMSO) 55.81 (CH_3), 111.87 (CH), 120.97 (CH), 121.57 (qC), 123.41 (qC), 126.61 (CH), 128.64 (CH), 132.44 (CH), 158.14 (qC), 167.46 (qC), 168.18 (qC) ppm; m/z (EI) 235 (M, 40 %) and 164 (M-C(O)NHC(O), 100); (Found M^+ (EI), 235.03063. $\text{C}_{11}\text{H}_9\text{NO}_3\text{S}$ requires 235.03032).

6.4.3 (Z)-5-(3-methoxybenzylidene)thiazolidine-2,4-dione 198.

General procedure 1, for 24 h, afforded the title compound as a yellow powder on recrystallisation from ethanol (2.86 g, 12.2 mmol, 81 %); mp 196-198 °C (From EtOH); R_F (EtOAc-hexane, 1:1) 0.39; ν_{\max} (KBr)/ cm^{-1} 3030 (olefinic C-H/Ph-H), 2809 (CH_3), 1732 ($\text{C}=\text{O}$), 1682 ($\text{C}=\text{O}$), 1603 (Ph), 1574 (Ph), 1490 (CH_3), 1381 (CH_3) and 771 (Ph-H); δ_H (200 MHz; [$^2\text{H}_6$] DMSO) 3.79 (3H, s, OCH_3), 7.09 (3H, m, Ph), 7.44 (1H, t, J 8.0, Ph), 7.75 (1H, s, olefinic H), 12.65 (1H, br s, NH) ppm; δ_C (63MHz; [$^2\text{H}_6$] DMSO) 55.39 (CH_3), 115.41 (CH), 116.39 (CH), 122.02 (CH),

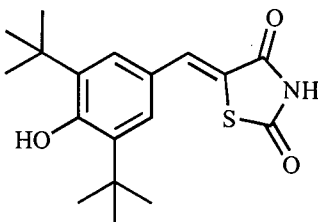
124.00 (qC), 130.53 (CH), 131.87 (CH), 134.49 (qC), 159.74 (qC), 167.58 (qC), 167.94 (qC) ppm; m/z (EI) 235 (M, 32 %) and 164 (M-C(O)NHC(O), 100); (Found M^+ (EI), 235.02929. $C_{11}H_9NO_3S$ requires 235.03032).

6.4.4 (Z)-5-(2,6-dimethoxybenzylidene)thiazolidine-2,4-dione 199.



General procedure 2, for 18 h, afforded the title compound as a yellow powder (1.31 g, 4.94 mmol, 82 %); mp 233-235 °C (From EtOH); R_f (EtOAc-hexane, 1:1) 0.44; ν_{\max} (KBr)/ cm^{-1} 3030 (olefinic C-H/Ph-H), 1727 (C=O), 1691 (C=O), 1588 (Ph), 1573 (Ph), 1478 (CH_3), 1386 (CH_3) and 777 (Ph-H); δ_H (200 MHz; $[\text{}^2\text{H}_6]$ DMSO) 3.85 (6H, s, 2OCH_3), 6.73 (2H, d, J 8.5, Ph), 7.43 (1H, t, J 8.5, Ph), 7.90 (1H, s, olefinic H) ppm; δ_C (50MHz; $[\text{}^2\text{H}_6]$ DMSO) 55.79 (CH_3), 104.22 (CH), 109.88 (qC), 125.37 (qC), 125.78 (CH), 133.24 (CH), 158.28 (qC), 167.99 (qC), 169.08 (qC) ppm; m/z (EI) 265 (M, 63 %) and 194 (M-C(O)NHC(O), 100); (Found M^+ (EI), 265.04069. $C_{12}H_{11}NO_4S$ requires 265.04088).

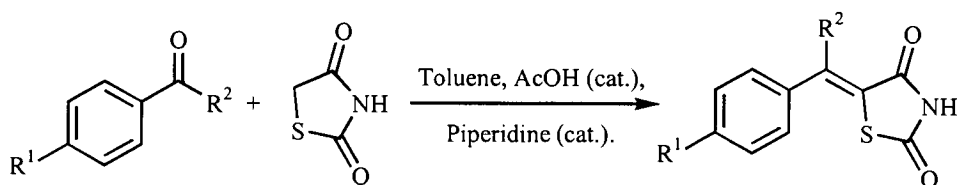
6.4.5 (Z)-5-(3,5-di-*t*-butyl-4-hydroxybenzylidene)thiazolidine-2,4-dione 200.



General procedure 1, for 36 h, afforded the title compound as a light brown powder on recrystallisation from toluene (3.99 g, 12.0 mmol, 80 %); mp 226-228 °C (From toluene) (lit.,¹⁷⁴ 238-240 °C (from EtOH)); R_f (EtOAc-hexane, 1:1) 0.47; ν_{\max}

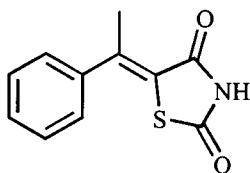
(KBr)/cm⁻¹ 2964 (CH₃), 1743 (C=O), 1687 (C=O), 1594 (Ph), 1474 (CH₃), 1361 (CH₃) and 808 (Ph-H) (lit.,¹⁷⁴); δ_H (250 MHz; [²H₆] DMSO) 1.39 (18H, s, 6CH₃), 7.34 (2H, s, Ph), 7.73 (1H, s, olefinic H), 12.48 (1H, br s, NH) ppm (lit.,¹⁷⁴); δ_C (63MHz; [²H₆] DMSO) 30.09 (CH₃), 34.72 (qC), 119.17 (qC), 124.45 (qC), 127.59 (CH), 133.54 (CH), 139.47 (qC), 156.67 (qC), 167.51 (qC), 168.15 (qC) ppm; *m/z* (EI) 333 (M, 36 %) and 262 (M-C(O)NHC(O), 100); (Found M⁺ (EI), 333.14034. C₁₈H₂₃NO₃S requires 333.13987).

6.5 Knoevenagel Condensation Between Thiazolidine-2,4-dione and 4-Substituted Benzophenones.

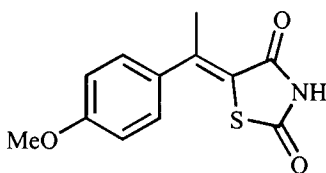


6.5.1 General Procedure.

Thiazolidine-2,4-dione (15.0 mmol) and the appropriate phenone (15.0 mmol) were heated at reflux in toluene (30 cm³), containing piperidine (0.45 mmol) and acetic acid (0.45 mmol), under Dean and Stark conditions,.

6.5.2 (Z)-5-(1-methyl-1-phenylmethylidene)thiazolidine-2,4-dione 201.

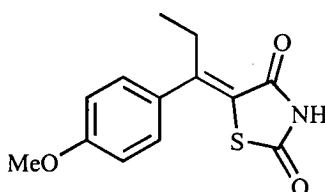
After 50 h the reaction mixture was allowed to cool and the crude product recovered by filtration and recrystallised from toluene. The mother liquors were reduced and recrystallised from toluene. Both sets of crystals were combined to afford the title compound as a light brown solid (1.65 g, 7.53 mmol, 52 %); mp 124-126 °C (From toluene); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.60; (Found : C, 60.06; H, 4.23; N, 6.40. $C_{11}H_9NO_2S$ requires C, 60.27; H, 4.11; N, 6.39 %); ν_{\max} (KBr)/ cm^{-1} 1737 (C=O), 1678 (C=O), 1582 (Ph), 1567 (Ph), 1489 (CH₃), 1377 (CH₃) and 767 (Ph-H); δ_H (250 MHz; [²H₆] DMSO) 2.64 (3H, s, CH₃), 7.43 (5H, m, Ph) ppm; δ_C (63MHz; [²H₆] DMSO) 21.62 (CH₃), 122.06 (qC), 126.76 (CH), 129.03 (CH), 129.41 (qC), 142.19 (qC), 149.61 (qC), 166.67 (qC), 167.72 (qC) ppm; m/z (EI) 219 (M, 52 %) and 148 (M-C(O)NHC(O), 100); (Found M^+ (EI), 219.03576. $C_{11}H_9NO_2S$ requires 219.03540).

6.5.3 (Z)-5-(1-methyl-1-(4-methoxyphenyl)methylidene)thiazolidine-2,4-dione 202.

After 47 h the reaction mixture was allowed to cool and the crude product recovered by filtration and recrystallised from toluene. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:3) as eluent, afforded the title compound as a light green/yellow powder (1.04 g, 4.20 mmol, 29 %); mp 155-156 °C; R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:3) 0.22; (Found : C, 57.87; H, 4.42; N, 5.62. $C_{12}H_{11}NO_3S$ requires C, 57.83; H, 4.42; N, 5.62 %); ν_{\max}

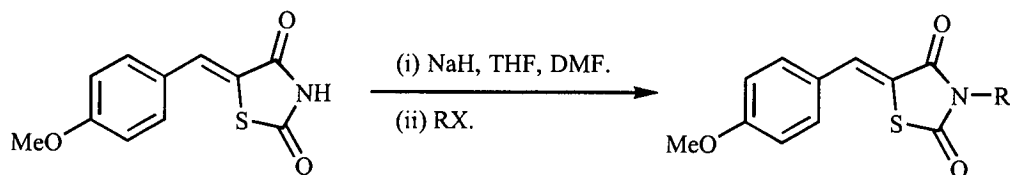
(KBr)/cm⁻¹ 2842 (CH₃), 2783 (CH₃), 1720 (C=O), 1675 (C=O), 1586 (Ph), 1560 (Ph), 1511 (Ph), 1463 (CH₃), 1367 (CH₃) and 837 (Ph-H); δ_H (250 MHz; [²H₆] DMSO) 2.62 (3H, s, CH₃), 3.78 (3H, s, OCH₃), 6.99 (2H, d, *J* 9.0, Ph), 7.37 (2H, d, *J* 9.0, Ph), 12.22 (1H, br s, NH) ppm; δ_C (63MHz; [²H₆] DMSO) 21.56 (CH₃), 55.38 (CH₃), 114.27 (CH), 120.95 (qC), 128.60 (CH), 134.16 (qC), 149.50 (qC), 160.02 (qC), 166.67 (qC), 167.87 (qC) ppm; *m/z* (EI) 249 (M, 88 %) and 178 (M-C(O)NHC(O), 100); (Found M⁺ (EI), 249.04574. C₁₂H₁₁NO₃S requires 249.04597).

6.5.4 (Z)-5-(1-ethyl-1-(4-methoxyphenyl)methylidene)thiazolidine-2,4-dione **203**.



After 10 d the reaction mixture was concentrated under reduced pressure. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (2:7) as eluent, afforded the title compound as a light yellow powder (1.89 g, 7.20 mmol, 50 %); mp 109-110 °C; *R_F* (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.52; (Found : C, 59.25; H, 5.05; N, 5.30. C₁₃H₁₃NO₃S requires C, 59.32; H, 4.94; N, 5.32 %); ν_{max} (KBr)/cm⁻¹ 2972 (CH₂/CH₃), 1724 (C=O), 1683 (C=O), 1578 (Ph), 1560 (Ph), 1510 (Ph), 1456 (CH₂/CH₃), 1372 (CH₃) and 840 (Ph-H); δ_H (250 MHz; [²H₆] DMSO) 0.93 (3H, t, *J* 7.5, CH₂CH₃), 3.14 (2H, q, *J* 7.5, CH₂CH₃), 3.78 (3H, s, OCH₃), 7.00 (2H, d, *J* 9.0, Ph), 7.32 (2H, d, *J* 9.0, Ph), 12.24 (1H, br s, NH) ppm; δ_C (63MHz; [²H₆] DMSO) 12.89 (CH₃), 27.25 (CH₂), 55.33 (CH₃), 113.28 (CH), 121.31 (qC), 128.78 (CH), 132.62 (qC), 155.90 (qC), 159.98 (qC), 166.21 (qC), 167.89 (qC) ppm; *m/z* (EI) 263 (M, 63 %) and 192 (M-C(O)NHC(O), 100); (Found M⁺ (EI), 263.06089. C₁₃H₁₃NO₃S requires 263.06162).

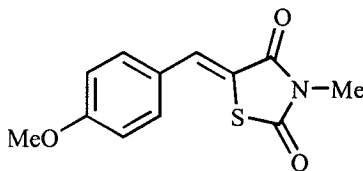
6.6 Synthesis Of (Z)-5-(4-substitutedbenzylidene)-3-substituted thiazolidine-2,4-diones.



6.6.1 General Procedure.

Sodium hydride, as a 60 % dispersion in mineral oil, (0.25 g, 6.2 mmol) was washed, under nitrogen, with hexane (2 x 10 cm³) and the resultant powder resuspended in THF (40 cm³) and DMF (10 cm³). **172** (1.30 g, 5.53 mmol) was added and the suspension heated to 40 °C. Iodomethane or benzylbromide (6.00 mmol) was added and the suspended material instantly dissolved, at which point the solution was cooled to ambient temperature. Water (50 cm³) was added, followed by sat. aqueous sodium chloride (300 cm³), and the mixture was extracted with ethyl acetate (3 x 150 cm³). The combined organics were washed with water (4 x 200 cm³), dried over sodium sulfate and evaporated under reduced pressure to afford the title compound.

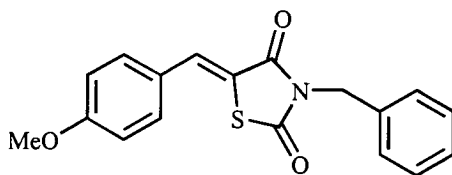
6.6.2 (Z)-3-methyl-5-(4-methoxybenzylidene)thiazolidine-2,4-dione **204**.



Using iodomethane in the general procedure afforded the title compound as a light green powder (1.38 g, 5.54 mmol, 100 %); mp 140-142 °C; R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.58; (Found : C, 58.04; H, 4.67; N, 5.63. C₁₂H₁₁NO₃S

requires C, 57.83; H, 4.42; N, 5.62 %); ν_{\max} (KBr)/ cm^{-1} 2941 (CH_3), 1731 ($\text{C}=\text{O}$), 1679 ($\text{C}=\text{O}$), 1592 (Ph), 1511 (Ph), 1434 (CH_3), 1365 (CH_3) and 830 (Ph-H); δ_{H} (250 MHz; [$^2\text{H}_6$] DMSO) 3.09 (3H, s, CH_3), 3.83 (3H, s, OCH_3), 7.10 (2H, d, J 9.0, Ph), 7.58 (2H, d, J 9.0, Ph), 7.86 (1H, s, olefinic H) ppm; δ_{C} (63MHz; [$^2\text{H}_6$] DMSO) 27.87 (CH_3), 55.61 (CH_3), 115.05 (CH), 118.34 (qC), 125.54 (qC), 132.31 (CH), 132.71 (CH), 161.21 (qC), 166.02 (qC), 167.54 (qC) ppm; m/z (EI) 249 (M, 52 %), 234 (M- CH_3 , 29) and 164 (M- $\text{C}(\text{O})\text{N}(\text{CH}_3)\text{C}(\text{O})$, 100); (Found M^+ (EI), 249.04605. $\text{C}_{12}\text{H}_{11}\text{NO}_3\text{S}$ requires 249.04597).

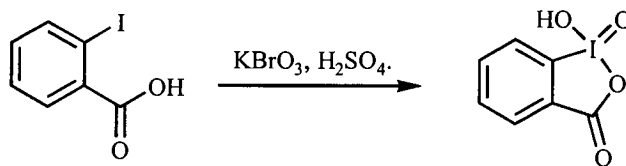
6.6.3 (Z)-3-benzyl-5-(4-methoxybenzylidene)thiazolidine-2,4-dione **205**.



Using benzylbromide in the general procedure afforded the title compound as a yellow powder (1.78 g, 5.48 mmol, 100 %); mp 145-147 °C; R_{F} (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.69; ν_{\max} (KBr)/ cm^{-1} 3012 (olefinic C-H/Ph-H), 2965 (CH_2/CH_3), 2930 (CH_2/CH_3), 1736 ($\text{C}=\text{O}$), 1673 ($\text{C}=\text{O}$), 1589 (Ph), 1565 (Ph), 1512 (Ph), 1454 (CH_2/CH_3), 1379 (CH_3), 829 (Ph-H), 746 (Ph-H), 726 (CH_2) and 690 (Ph-H); δ_{H} (250 MHz; [$^2\text{H}_6$] DMSO) 3.78 (3H, s, OCH_3), 4.83 (2H, s, CH_2Ph), 7.11 (2H, d, J 9.0, Ph), 7.32 (5H, m, Ph), 7.60 (2H, d, J 9.0, Ph), 7.92 (1H, s, olefinic H) ppm; δ_{C} (63MHz; [$^2\text{H}_6$] DMSO) 44.68 (CH_2), 55.63 (CH_3), 115.12 (CH), 117.89 (qC), 125.49 (qC), 127.71 (CH), 127.90 (CH), 128.78 (CH), 132.46 (CH), 133.64 (CH), 135.67 (qC), 161.39 (qC), 165.75 (qC), 167.50 (qC) ppm; m/z (EI) 325 (M, 18 %) and 164 (M- $\text{C}(\text{O})\text{N}(\text{CH}_2\text{Ph})\text{C}(\text{O})$, 35); (Found M^+ (EI), 325.07775. $\text{C}_{18}\text{H}_{15}\text{NO}_3\text{S}$ requires 325.07727).

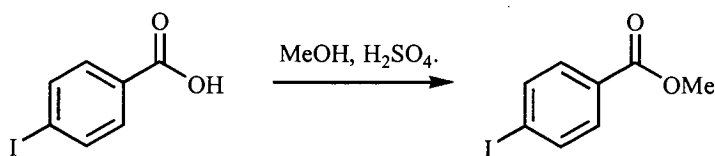
6.7 Synthesis of (Z)-5-(4-iodobenzylidene)thiazolidine-2,4-dione.

6.7.1 2-Iodoxy benzoic acid **210**.¹⁴⁵



Potassium bromate (22.3 g, 133 mmol) was added over 30 min to a vigorously stirred mixture of 2-iodobenzoic acid (25.1 g, 101 mmol) in 0.73 M sulfuric acid (215 cm³) at 55 °C. The heterogeneous mixture was heated to 68 °C and stirred for 3.5 h before being cooled on ice. The resultant solid was recovered by filtration and washed successively with water (2 x 250 cm³), acetone (250 cm³) and diethyl ether (250 cm³) before being dried under vacuum to afford the title compound as a yellow powder (24.6 g, 88 mmol, 88 %); δ_{H} (250 MHz; [²H₆] DMSO) 7.84 (1H, t, *J* 7.0, Ph), 8.01 (2H, m, Ph), 8.14 (1H, d, *J* 8.0, Ph) ppm (lit.,¹⁷⁸); δ_{C} (63MHz; [²H₆] DMSO) 125.13 (CH), 130.23 (CH), 131.54 (qC), 133.10 (CH), 133.55 (CH), 146.67 (qC), 167.65 (qC) ppm; *m/z* (EI) 280 (M, 3 %), 248 (M-O₂, 100), 231 (M-HO₃, 99), 203 (M-CHO₄, 41) and 76 (M-CHO₄I, 37).

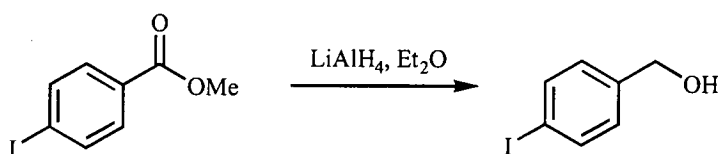
6.7.2 4-Iodobenzoic acid methyl ester **207**.



4-Iodobenzoic acid **206** (12.5 g, 50.0 mmol) was heated at reflux in methanol (150 cm³), containing 98 % sulfuric acid (1.2 cm³), for 15.5 h. After cooling the solvent was removed under reduced pressure and the crude product dissolved in ethyl acetate

(500 cm³). This was washed successively with sat. aqueous sodium hydrogen carbonate (2 x 100 cm³), sat. aqueous sodium chloride (100 cm³) and water (100 cm³). The organics were dried over magnesium sulfate, filtered, and the solvent removed under reduced pressure. Recrystallisation from methanol afforded the title compound as white crystals. (5.54 g, 33.8 mmol, 94 %); mp 113-115 °C (From MeOH) (lit.,¹⁷⁹ 115.4-115.7 °C); *R*_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.79; ν_{max} (Film)/cm⁻¹ 2979 (CH₃), 1719 (C=O), 1586 (Ph), 1452 (CH₃), 1392 (CH₃) and 844 (Ph-H); δ_{H} (250 MHz; CDCl₃) 3.89 (3H, s, OCH₃), 7.98 (4H, s, Ph) ppm (lit.,¹⁷⁹); δ_{C} (63MHz; CDCl₃) 52.14 (CH₃), 100.58 (qC), 129.43 (qC), 130.86 (CH), 137.55 (CH), 166.41 (qC) ppm; *m/z* (EI) 262 (M, 100 %), 231 (M-OCH₃, 94) and 203 (M-CO₂CH₃, 24); (Found *M*⁺ (EI), 261.94916. C₈H₇O₂I requires 261.94908).

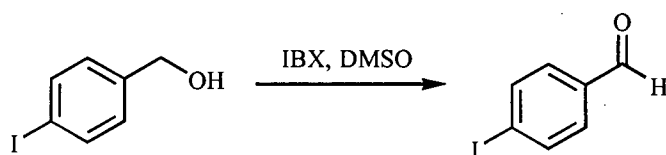
6.7.3 4-Iodobenzyl alcohol **208**.



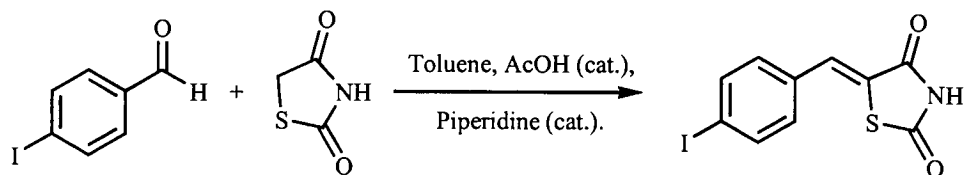
Lithium aluminium hydride (1.91 g, 50.3 mmol) in anhydrous diethyl ether (75 cm³) was cooled, under argon, in an ice bath. Ester **207** (11.9 g, 45.2 mmol) was added in portions as a solid over 20 min. Care: Effervescence! After the final addition the suspension was allowed to warm to ambient temperature and stirred for a further 1 h. Ethyl acetate (100 cm³) was added followed by 1 M hydrochloric acid (100 cm³) before filtration through Celite™. The aqueous layer was separated and the organics washed successively with sat. aqueous sodium hydrogen carbonate (100 cm³) and sat. aqueous sodium chloride (100 cm³). The aqueous layer was washed with ethyl acetate (2 x 100 cm³). The organics were combined, washed with water (2 x 100 cm³) dried over magnesium sulfate and the solvent removed under reduced pressure. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:4) as eluent afforded the title compound as a white powder

(9.25 g, 39.5 mmol, 88 %); mp 73-74 °C (lit.,¹⁸⁰ 71.8-73.4 °C); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.49; ν_{\max} (Film)/ cm^{-1} 3307 (O-H), 2922 (CH_2), 2868 (CH_2), 1587 (Ph), 1484 (CH_2), 1410 (O-H), 841 (Ph-H) and 748 (CH_2); δ_H (250 MHz; CDCl_3) 2.55 (1H, br s, OH), 4.54 (2H, s, CH_2OH), 7.04 (2H, d, J 8.5, Ph), 7.64 (2H, d, J 8.5, Ph) ppm (lit.,^{180,181}); δ_C (63MHz; CDCl_3) 64.29 (CH_2), 92.76 (qC), 128.60 (CH), 137.38 (CH), 140.25 (qC) ppm; m/z (EI) 234 (M, 100 %), 217 (M-OH, 7) and 203 (M- CH_2OH , 6); (Found M^+ (EI), 233.95467. $\text{C}_7\text{H}_7\text{OI}$ requires 233.95417).

6.7.4 4-Iodobenzaldehyde **209**.¹⁷⁸



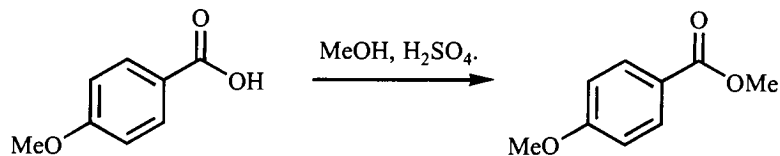
Alcohol **208** (6.09 g, 26.0 mmol) was added to a solution of IBX (7.90 g, 28.2 mmol) in DMSO (30 cm^3). The mixture turned yellow and was stirred at ambient temperature for 15 min, during which time a precipitate had formed. The reaction was then quenched with water (100 cm^3) and the suspension filtered through Celite™. The filtrate was extracted with ethyl acetate (2 x 200 cm^3). The organics were then combined and washed with water (200 cm^3), dried over magnesium sulfate, filtered, and the solvent removed under reduced pressure. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:1) as eluent to afford the title compound as an orange powder (4.53g, 19.5 mmol, 75 %); mp 76-77 °C (lit.,¹⁸² 65-68 °C); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.79; ν_{\max} (Film)/ cm^{-1} 2827 (CH_2), 1688 (C=O), 1584 (Ph) and 804 (Ph-H) (lit.,¹⁸³); δ_H (250 MHz; CDCl_3) 7.55 (2H, d, J 8.5, Ph), 7.87 (2H, d, J 8.5, Ph) 9.92 (1H, s, C(O)H) ppm (lit.,¹⁸²); δ_C (63MHz; CDCl_3) 102.67 (qC), 130.60 (CH), 135.32 (qC), 138.18 (CH), 191.22 (CH) ppm; m/z (EI) 232 (M, 100 %) and 203 (M-C(O)H, 42); (Found M^+ (EI), 231.93863. $\text{C}_7\text{H}_5\text{OI}$ requires 231.93852).

6.7.5 (Z)-5-(4-iodobenzylidene)thiazolidine-2,4-dione 188.

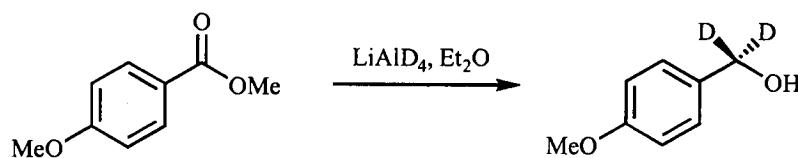
Thiazolidine-2,4-dione (1.76 g, 15.0 mmol) and aldehyde **209** (3.48 g, 15.0 mmol) were heated at reflux in toluene (100 cm³), containing piperidine (36 mg, 0.42 mmol) and acetic acid (30 mg, 0.50 mmol), under Dean and Stark conditions for 30 h.. The solution was allowed to cool and the precipitate recovered by filtration and recrystallised from ethanol. The mother liquors were reduced and recrystallised to obtain a second crop of crystals. This afforded the title compound as a yellow powder (1.68 g, 5.08 mmol, 34 %); mp 232-234 °C (From EtOH); R_f (EtOAc-hexane, 1:1) 0.57; ν_{\max} (KBr)/cm⁻¹ 3052 (olefinic C-H/Ph-H), 1749 (C=O), 1717 (C=O), 1610 (Ph), 1578 (Ph), 807 (Ph-H) and 516 (C-I); δ_H (200 MHz; [²H₆] DMSO) 7.37 (2H, d, J 8.5, Ph), 7.87 (1H, s, olefinic H) 7.90 (2H, d, J 8.5, Ph), 12.66 (1H, br s, NH) ppm; δ_C (50MHz; [²H₆] DMSO) 97.87 (qC), 124.43 (qC), 130.86 (CH), 131.71 (CH), 132.58 (qC), 138.25 (CH), 167.29 (qC), 167.68 (qC) ppm; m/z (EI) 331 (M, 68 %) and 260 (M-C(O)NHC(O), 72); (Found M^+ (EI), 330.91634. C₁₀H₆NO₂SI requires 330.91640).

6.8 Synthesis Of (Z)-5-(4-methoxybenzylidene-*d*)thiazolidine-2,4-dione.

6.8.1 4-Methoxy benzoic acid methyl ester **212**.

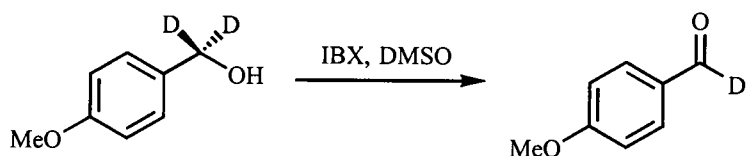


4-Methoxybenzoic acid **211** (5.20 g, 34.2 mmol) was heated at reflux in methanol (100 cm³), containing 98 % sulfuric acid (1.5 cm³), for 22 h. After cooling the solvent was removed under reduced pressure and the crude product dissolved in ethyl acetate (150 cm³). This was washed successively with sat. aqueous sodium hydrogen carbonate (2 x 50 cm³), sat. aqueous sodium chloride (50 cm³) and water (50 cm³). The organics were dried over magnesium sulfate, filtered, and the solvent removed under reduced pressure to afford the title compound as white crystals (5.54 g, 33.8 mmol, 99 %); mp 51-53 °C; *R_F* (EtOAc-petroleum ether (bp 40-65 °C), 2:1) 0.89; ν_{max} (Film)/cm⁻¹ 3002 (Ph-H), 2967 (CH₃), 2844 (CH₃), 1711 (C=O), 1610 (Ph), 1513 (Ph), 1446 (CH₃), 1432 (CH₃) and 848 (Ph-H); δ_{H} (250 MHz; CDCl₃) 3.84 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 6.90 (2H, d, *J* 9.0, Ph), 7.98 (2H, d, *J* 9.0, Ph) ppm; δ_{C} (63MHz; CDCl₃) 51.75 (CH₃), 55.29 (CH₃), 113.46 (CH), 122.45 (qC), 131.45 (CH), 163.18 (qC), 166.75 (qC) ppm; *m/z* (EI) 166 (M, 59 %), 135 (M-OCH₃, 100) and 107 (M-CO₂CH₃, 42); (Found *M*⁺ (EI), 166.06303. C₉H₁₀O₃ requires 166.06300).

6.8.2 4-Methoxy benzyl alcohol-*d*₂ **213**.

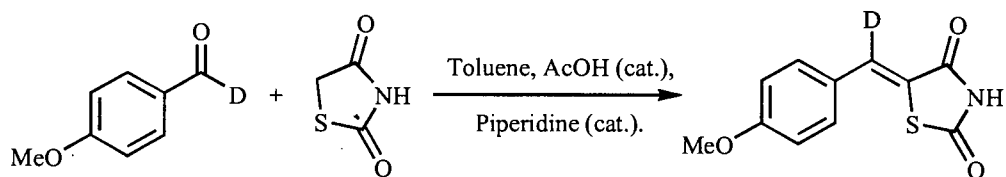
Lithium aluminium deuteride (1.40 g, 33.3 mmol) in anhydrous diethyl ether (10 cm³) was cooled, under argon, in an ice bath. A solution of ester **212** (5.00 g, 30.5 mmol) in anhydrous diethyl ether (50 cm³) was added dropwise over 10 min. The solution was allowed to stir on ice for 2 h before warming to ambient temperature and stirring for a further 2 h. Ethyl acetate (100 cm³) was added followed by 1 M hydrochloric acid (75 cm³) before filtration through Celite™. The aqueous layer was separated and the organics washed successively with sat. aqueous sodium hydrogen carbonate (75 cm³), sat. aqueous sodium chloride (75 cm³) and water (75 cm³). The organics were dried over magnesium sulfate and the solvent removed under reduced pressure. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (3:1), increasing to ethyl acetate, as eluent, afforded the title compound as a light yellow oil (3.01 g, 21.5 mmol, 68 %); mp 23-24 °C (lit.,¹⁸⁴ 25 °C); *R*_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.56; ν_{max} (Film)/cm⁻¹ 3356 (O-H), 3002 (Ph-H), 2956 (CH₃), 2908 (CH₃), 2835 (CH₃), 2177 (CD₂), 2138 (CD₂), 2084 (CD₂), 1464 (CH₃), 1371 (CH₃) and 845 (Ph-H) (lit.,¹⁸⁵); δ_{H} (250 MHz; CDCl₃) 1.86 (1H, br s, OH), 3.79 (3H, s, OCH₃), 6.88 (2H, d, *J* 9.0, Ph), 7.27 (2H, d, *J* 9.0, Ph) ppm (lit.,^{185,186}); δ_{C} (63MHz; CDCl₃) 55.14 (CH₃), 64.13 (tt, *J* 22, CD₂), 113.77 (CH), 128.53 (CH), 132.85 (qC), 159.02 (qC) ppm (lit.,¹⁸⁶); *m/z* (EI) 140 (M, 100 %), 123 (M-OH, 89) and 107 (M-CD₂OH, 21); (Found *M*⁺ (EI), 140.08109. C₈H₈D₂O₂ requires 140.08063).

6.8.3 4-Methoxy benzaldehyde-*d* **214**.¹⁷⁸



A solution of alcohol **213** (2.50 g, 17.9 mmol) in DMSO (10 cm³) was added to a solution of IBX (5.50 g, 19.6 mmol) in DMSO (20 cm³). The mixture turned yellow and was stirred at ambient temperature for 45 min, during which time a precipitate had formed. The reaction was then quenched with water (100 cm³) and the suspension filtered through Celite™. The filtrate was extracted with ethyl acetate (3 x 100 cm³). The organics were dried over magnesium sulfate, filtered, and the solvent removed under reduced pressure. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:1) as eluent, afforded the title compound as a light yellow oil (2.17 g, 15.8 mmol, 88 %); *R*_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.73; ν_{max} (Film)/cm⁻¹ 3002 (Ph-H), 2967 (CH₃), 2844 (CH₃), 1711 (C=O), 1446 (CH₃) and 848 (Ph-H) (lit.,^{184,187,188}); δ_{H} (250 MHz; CDCl₃) 3.87 (3H, s, OCH₃), 6.99 (2H, d, *J* 9.0, Ph), 7.83 (2H, d, *J* 9.0, Ph) ppm (lit.,^{189,190}); δ_{C} (63MHz; CDCl₃) 55.44 (CH₃), 114.17 (CH), 129.65 (qC), 131.85 (CH), 164.47 (qC), 190.42 (t, *J* 27, C(O)D) ppm; *m/z* (EI) 137 (M, 82 %) and 107 (M-C(O)D, 52); (Found *M*⁺ (EI), 137.05898. C₈H₇DO₂ requires 137.05871).

6.8.4 (*Z*)-5-(4-methoxybenzylidene-*d*)thiazolidine-2,4-dione **215**.

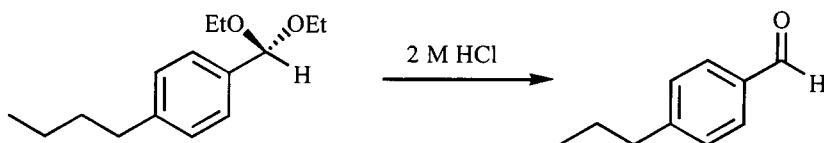


Thiazolidine-2,4-dione (1.19 g, 10.2 mmol) and aldehyde **214** (1.90 g, 13.9 mmol) were heated at reflux in toluene (100 cm³), containing piperidine (50 μ l) and acetic

acid (50 μ l), under Dean and Stark conditions for 12 h. The solution was cooled and the precipitate recovered by filtration and recrystallised from ethanol. The mother liquors were reduced and recrystallised to obtain a second crop of crystals. This afforded the title compound as a yellow powder (1.95 g, 8.3 mmol, 81 %); mp 218-219 °C (From EtOH); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.50; ν_{\max} (KBr)/ cm^{-1} 3002 (Ph-H), 2778 (CH_3), 1731 ($\text{C}=\text{O}$), 1696 ($\text{C}=\text{O}$), 1584 (Ph), 1509 (Ph), 1470 (CH_3) and 830 (Ph-H); δ_H (250 MHz; [$^2\text{H}_6$] DMSO) 3.82 (3H, s, OCH_3), 7.09 (2H, d, J 9.0, Ph), 7.55 (2H, d, J 9.0, Ph), 12.52 (1H, br s, NH) ppm; δ_C (63MHz; [$^2\text{H}_6$] DMSO) 55.60 (CH_3), 115.03 (CH), 120.28 (qC), 125.53 (qC), 132.21 (CH), 161.10 (qC), 167.57 (qC), 168.09 (qC) ppm; m/z (EI) 236 (M, 91 %) and 165 (M-C(O)NHC(O), 100); (Found M^+ (EI), 236.03692. $\text{C}_{11}\text{H}_8\text{DNO}_3\text{S}$ requires 236.03660).

6.9 Synthesis of (Z)-5-(4-propylbenzylidene)thiazolidine-2,4-dione.

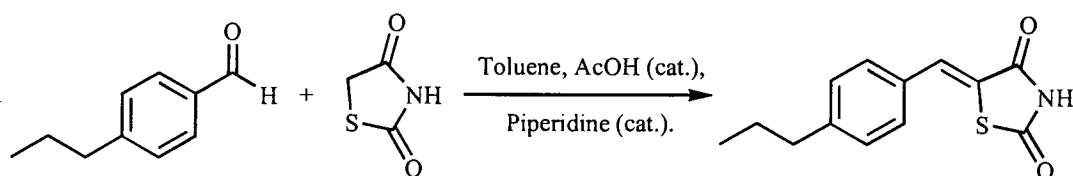
6.9.1 4-Propylbenzaldehyde 216.



4-Propylbenzaldehyde diethylacetate (2.33 g, 10.5 mmol) was stirred at ambient temperature for 1 h in 2 M hydrochloric acid (21.5 cm^3). The reaction was quenched by neutralisation with sat. aqueous sodium hydrogen carbonate before ethyl acetate (100 cm^3) was added. The layers were separated and the aqueous layer washed with ethyl acetate (100 cm^3). The organics were combined and washed successively with sat. aqueous sodium hydrogen carbonate (100 cm^3) and water (2 x 100 cm^3) before being dried with magnesium sulfate. The solvent was removed under reduced pressure to afford the title compound as a clear oil (1.30 g, 8.78 mmol, 84 %); R_F

(EtOAc-petroleum ether (bp 40-65 °C, 1:4) 0.50; ν_{\max} (Film)/cm⁻¹ 3055 (Ph-H), 2963 (CH₂/CH₃), 2934 (CH₂/CH₃), 2872 (CH₂/CH₃), 1693 (C=O), 1608 (Ph), 1575 (Ph), 1515 (Ph), 1465 (CH₂/CH₃), 824 (Ph-H) and 740 (CH₂); δ_{H} (250 MHz; CDCl₃) 0.91 (3H, t, *J* 7.5, CH₂CH₂CH₃), 1.64 (2H, tq, *J* 7.5, CH₂CH₂CH₃), 2.63 (2H, t, *J* 7.5, CH₂CH₂CH₃), 7.30 (2H, d, *J* 8.5, Ph), 7.76 (2H, d, *J* 8.5, Ph), 9.93 (1H, s, C(O)H) ppm; δ_{C} (63MHz; CDCl₃) 13.52 (CH₃), 23.97 (CH₂), 37.98 (CH₂), 128.91 (CH), 129.64 (CH), 134.20 (qC), 149.98 (qC), 191.82 (qC) ppm (lit.,¹⁹¹); *m/z* (EI) 148 (M, 10 %) and 119 (M-C(O)H, 10); (Found M⁺ (EI), 148.08904. C₁₀H₁₂O requires 148.08882).

6.9.2 (Z)-5-(4-propylbenzylidene)thiazolidine-2,4-dione **182**.

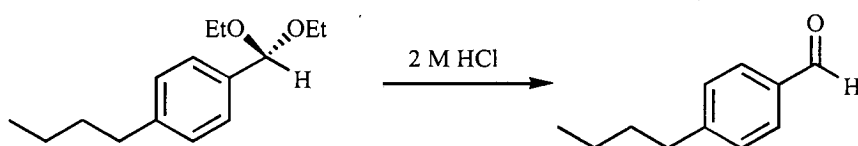


Thiazolidine-2,4-dione (817 mg, 6.99 mmol) and aldehyde **216** (1.03 g, 6.96 mmol) were heated at reflux in toluene (60 cm³), containing piperidine (16 mg, 0.19 mmol) and acetic acid (19 mg, 0.32 mmol), under Dean and Stark conditions for 22 h. The solution was allowed to cool to ambient temperature before the solvent was removed under reduced pressure. The resultant solid was recrystallised from ethanol. The mother liquors were reduced and recrystallised to obtain a second crop of crystals. This afforded the title compound as a yellow powder (1.57 g, 6.4 mmol, 90 %); mp 163-164 °C (From EtOH); *R_F* (EtOAc-hexane, 1:1) 0.47; ν_{\max} (KBr)/cm⁻¹ 3024 (olefinic C-H/Ph-H), 2928 (CH₂/CH₃), 2856 (CH₂/CH₃), 1739 (C=O), 1690 (C=O), 1600 (Ph), 1510 (Ph), 1463 (CH₂/CH₃), 1390 (CH₃), 824 (Ph-H) and 735 (CH₂); δ_{H} (250 MHz; [²H₆] DMSO) 0.85 (3H, t, *J* 7.5, CH₂CH₂CH₃), 1.56 (2H, tq, *J* 7.5, CH₂CH₂CH₃), 2.55 (2H, t, *J* 7.5, CH₂CH₂CH₃), 7.29 (2H, d, *J* 8.0, Ph), 7.45 (2H, d, *J* 8.0, Ph), 7.71 (1H, s, olefinic *H*) ppm; δ_{C} (63MHz; [²H₆] DMSO) 13.78 (CH₃), 23.95

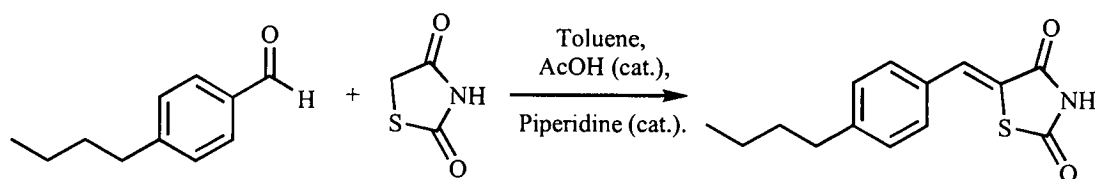
(CH₂), 37.30 (CH₂), 122.48 (qC), 129.48 (CH), 130.29 (CH), 130.70 (qC), 132.10 (CH), 145.43 (qC), 167.59 (qC), 168.14 (qC) ppm; *m/z* (EI) 247 (M, 67 %) and 176 (M-C(O)NHC(O), 91); (Found *M*⁺ (EI), 247.06746. C₁₃H₁₃NO₂S requires 247.06670).

6.10 Synthesis of (Z)-5-(4-butylbenzylidene)thiazolidine-2,4-dione.

6.10.1 4-Butylbenzaldehyde 217.



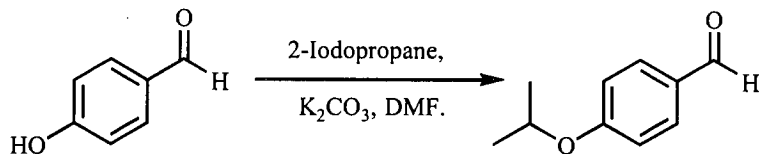
4-Butylbenzaldehyde diethylacetal (3.54 g, 15.0 mmol) was stirred at ambient temperature for 1 h in 2 M hydrochloric acid (31 cm³). The reaction was quenched by neutralisation with sat. aqueous sodium hydrogen carbonate before ethyl acetate (100 cm³) was added. The layers were separated and the aqueous layer washed with ethyl acetate (100 cm³). The organics were combined and washed successively with sat. aqueous sodium hydrogen carbonate (100 cm³) and water (2 x 100 cm³) before being dried with magnesium sulfate. The solvent was removed under reduced pressure to afford the title compound as a clear oil (2.32 g, 14.3 mmol, 95 %); *R_F* (EtOAc-petroleum ether (bp 40-65 °C, 1:7) 0.59; *ν*_{max} (Film)/cm⁻¹ 2954 (CH₂/CH₃), 2933 (CH₂/CH₃), 2860 (CH₂/CH₃), 1694 (C=O), 1606 (Ph), 1576 (Ph), 1516 (Ph), 1466 (CH₂/CH₃), 1380 (CH₃), 826 (Ph-H) and 740 (CH₂) (lit.,¹⁹²); *δ*_H (250 MHz; CDCl₃) 0.90 (3H, t, *J* 7.5, CH₂CH₂CH₂CH₃), 1.33 (2H, tq, *J* 7.5, CH₂CH₂CH₂CH₃), 1.59 (2H, tt, *J* 7.5, CH₂CH₂CH₂CH₃), 2.65 (2H, t, *J* 7.5, CH₂CH₂CH₂CH₃), 7.29 (2H, d, *J* 8.5, Ph), 7.76 (2H, d, *J* 8.5, Ph), 9.93 (1H, s, C(O)H) ppm (lit.,¹⁹²); *δ*_C (63MHz; CDCl₃) 13.66 (CH₃), 22.09 (CH₂), 32.97 (CH₂), 35.66 (CH₂), 128.85 (CH), 129.65 (CH), 134.16 (qC), 150.22 (qC), 191.79 (qC) ppm; *m/z* (EI) 162 (M, 56 %) and 133 (M-C(O)H, 6); (Found *M*⁺ (EI), 162.10458. C₁₁H₁₄O requires 162.10447).

6.10.2 (Z)-5-(4-butylbenzylidene)thiazolidine-2,4-dione **183**.

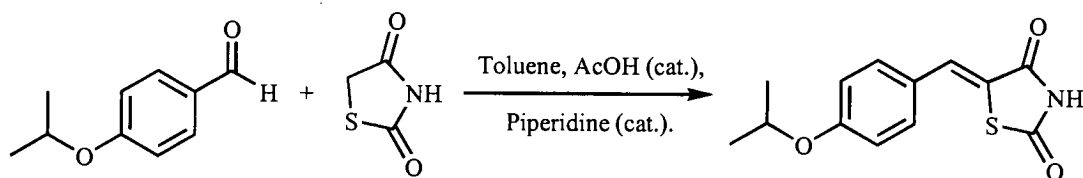
Thiazolidine-2,4-dione (1.45 g, 12.4 mmol) and aldehyde **217** (2.01 g, 12.4 mmol) were heated at reflux in toluene (60 cm³), containing piperidine (30 mg, 0.35 mmol) and acetic acid (25 mg, 0.42 mmol), under Dean and Stark conditions for 22 h. The solution was allowed to cool before the solvent was removed under reduced pressure. The resultant solid was recrystallised from ethanol. The mother liquors were reduced and recrystallised to obtain a second crop of crystals. This afforded the title compound as a yellow powder (2.85 g, 10.9 mmol, 88 %); mp 159-160 °C (From EtOH); *R_F* (EtOAc-hexane, 1:1) 0.52; (Found : C, 64.16; H, 5.94; N, 5.47. C₁₄H₁₅NO₂S requires C, 64.35; H, 5.79; N, 5.36 %); ν_{\max} (KBr)/cm⁻¹ 3024 (olefinic C-H/Ph-H), 2926 (CH₂/CH₃), 2858 (CH₂/CH₃), 1739 (C=O), 1693 (C=O), 1598 (Ph), 1508 (Ph), 1468 (CH₂/CH₃), 1384 (CH₃), 823 (Ph-H) and 749 (CH₂); δ_{H} (250 MHz; [²H₆] DMSO) 0.84 (3H, t, *J* 7.5, CH₂CH₂CH₂CH₃), 1.24 (2H, tq, *J* 7.5, CH₂CH₂CH₂CH₃), 1.49 (2H, tt, *J* 7.5, CH₂CH₂CH₂CH₃), 2.54 (2H, t, *J* 7.5, CH₂CH₂CH₂CH₃), 7.25 (2H, d, *J* 8.0, Ph), 7.41 (2H, d, *J* 8.0, Ph), 7.69 (1H, s, olefinic *H*) ppm; δ_{C} (63MHz; [²H₆] DMSO) 13.92 (CH₃), 22.01 (CH₂), 32.95 (CH₂), 34.98 (CH₂), 122.45 (qC), 129.37 (CH), 130.29 (CH), 130.66 (qC), 132.09 (CH), 145.60 (qC), 167.58 (qC), 168.11 (qC) ppm; *m/z* (EI) 261 (M, 41 %) and 190 (M-C(O)NHC(O), 67); (Found M⁺ (EI), 261.08335. C₁₄H₁₅NO₂S requires 261.08235).

6.11 Synthesis of (Z)-5-(4-*i*-propoxybenzylidene)thiazolidine-2,4-dione.

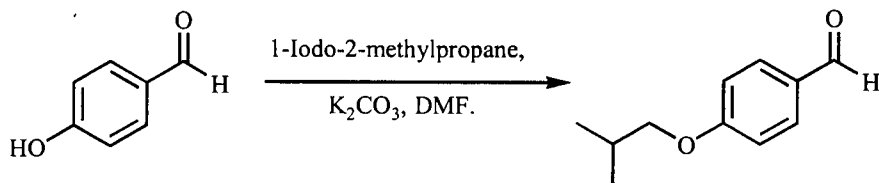
6.11.1 4-*i*-Propoxybenzaldehyde 218.



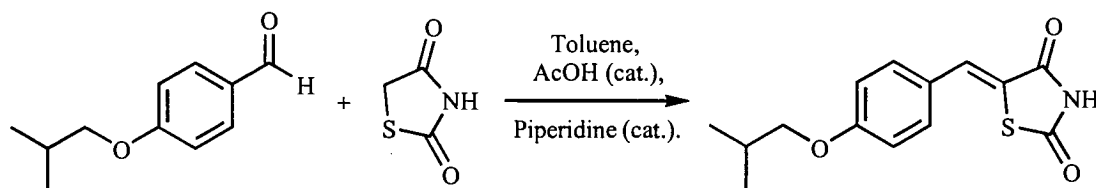
4-Hydroxybenzaldehyde (653 mg, 5.35 mmol), 2-iodopropane (1.00 g, 5.89 mmol) and potassium carbonate (813 mg, 5.89 mmol) were heated at 55 °C in DMF (40 cm³) for 96 h. The reaction mixture was allowed to cool before the solvent was removed under reduced pressure. The crude product was partitioned between water (50 cm³) and ethyl acetate (100 cm³). The layers were separated and the aqueous fraction washed with ethyl acetate (2 x 100 cm³). The organics were combined and washed successively with sat. aqueous sodium hydrogen carbonate (100 cm³), sat. aqueous sodium chloride (100 cm³) and water (100 cm³) before being dried over magnesium sulfate. The solvent was removed under reduced pressure and purification by column chromatography on silica gel, with ethyl acetate-hexane (1:3) as eluent, afforded the title compound as a light yellow oil (598 mg, 3.65 mmol, 68 %); R_F (EtOAc-hexane, 1:2) 0.58; ν_{\max} (Film)/cm⁻¹ 3073 (Ph-H), 2979 (CH₃), 2936 (CH₃), 2827 (CH₃), 1692 (C=O), 1601 (Ph), 1575 (Ph), 1505 (Ph), 1467 (CH₃), 1386 (CH₃) and 833 (Ph-H); δ_H (200 MHz; CDCl₃) 1.31 (6H, d, J 6.0, OCH(CH₃)₂), 4.61 (1H, spt, J 6.0, OCH(CH₃)₂), 6.91 (2H, d, J 9.0, Ph), 7.53 (2H, d, J 9.0, Ph), 9.80 (1H, s, C(O)H) ppm; δ_C (63MHz; CDCl₃) 21.60 (CH₃), 70.06 (CH), 115.35 (CH), 129.29 (qC), 131.74 (CH), 162.93 (qC), 190.44 (qC) ppm; m/z (EI) 164 (M, 14 %) and 121 (M-C₃H₇, 100); (Found M^+ (EI), 164.08373. C₁₀H₁₂O₂ requires 164.08373).

6.11.2 (Z)-5-(4-*i*-propoxybenzylidene)thiazolidine-2,4-dione **176**.

Thiazolidine-2,4-dione (339 mg, 2.90 mmol) and aldehyde **218** (476 mg, 2.90 mmol) were heated at reflux in toluene (20 cm³), containing piperidine (1 drop) and acetic acid (1 drop), under Dean and Stark conditions for 18 h. The solution was allowed to cool and the precipitate recovered by filtration. Recrystallised from ethanol afforded the title compound as a yellow powder (336 mg, 1.28 mmol, 44 %); mp 201-203 °C (From EtOH); *R_F* (EtOAc-hexane, 1:1) 0.59; (Found : C, 59.22; H, 5.03; N, 5.28. C₁₃H₁₃NO₃S requires C, 59.30; H, 4.98; N, 5.32 %); ν_{\max} (KBr)/cm⁻¹ 2973 (CH₃), 2930 (CH₃), 1736 (C=O), 1688 (C=O), 1592 (Ph), 1567 (Ph), 1511 (Ph), 1435 (CH₃), 1385 (CH₃) and 830 (Ph-H); δ_{H} (200 MHz; [²H₆] DMSO) 1.28 (6H, d, *J* 6.0, OCH(CH₃)₂), 4.72 (1H, spt, *J* 6.0, OCH(CH₃)₂), 7.06 (2H, d, *J* 9.0, Ph), 7.53 (2H, d, *J* 9.0, Ph), 7.74 (1H, s, olefinic CH), 10.73 (1H, br s, NH) ppm; δ_{C} (50MHz; [²H₆] DMSO) 21.78 (CH₃), 69.75 (CH), 116.24 (CH), 120.12 (qC), 125.18 (qC), 132.00 (CH), 132.27 (CH), 159.44 (qC), 167.56 (qC), 168.06 (qC) ppm; ; *m/z* (EI) 263 (M, 24 %), 221 (M-C₃H₇, 25), 192 (M-C(O)NHC(O), 2) and 150 (M-C₃H₇-C(O)NHC(O), 100); (Found M⁺ (EI), 263.06095. C₁₃H₁₃NO₃S requires 263.06162).

6.12 Synthesis of (Z)-5-(4-*i*-butoxybenzylidene)thiazolidine-2,4-dione.6.12.1 4-*i*-Butoxybenzaldehyde 219.

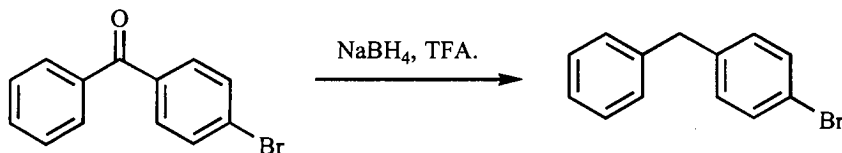
4-Hydroxybenzaldehyde (2.44 g, 20.0 mmol), 1-iodo-2-methylpropane (4.05 g, 22.0 mmol) and potassium carbonate (3.04 g, 22.0 mmol) were heated at 55 °C in DMF (148 cm³) for 120 h. The reaction mixture was allowed to cool before the solvent was removed under reduced pressure. The crude product was partitioned between water (50 cm³) and ethyl acetate (150 cm³). The layers were separated and the aqueous fraction washed with ethyl acetate (3 x 150 cm³). The organics were combined and washed successively with sat. aqueous sodium hydrogen carbonate (100 cm³), sat. aqueous sodium chloride (100 cm³) and water (100 cm³) before being dried over magnesium sulfate. The solvent was removed under reduced pressure and purification by column chromatography on silica gel, with ethyl acetate-hexane (1:9) as eluent, afforded the title compound as a light yellow oil (849 mg, 4.77 mmol, 22 %); *R*_F (EtOAc-hexane, 1:1) 0.44; ν_{max} (Film)/cm⁻¹ 3075 (Ph-H), 2961 (CH₂/CH₃), 2933 (CH₂/CH₃), 2874 (CH₂/CH₃), 1694 (C=O), 1601 (Ph), 1578 (Ph), 1511 (Ph), 1471 (CH₂/CH₃), 1396 (CH₃) and 832 (Ph-H); δ_{H} (250 MHz; CDCl₃) 0.99 (6H, d, *J* 6.5, OCH₂CH(CH₃)₂), 2.05 (1H, tspt, *J* 6.5, OCH₂CH(CH₃)₂), 3.74 (2H, d, *J* 6.5, OCH₂CH(CH₃)₂), 6.93 (2H, d, *J* 9.0, Ph), 7.76 (2H, d, *J* 9.0, Ph), 9.81 (1H, s, C(O)H) ppm; δ_{C} (63MHz; CDCl₃) 18.87 (CH₃), 27.92 (CH), 74.40 (CH₂), 114.51 (CH), 129.51 (qC), 131.67 (CH), 164.10 (qC), 190.45 (qC) ppm; *m/z* (EI) 178 (M, 21 %) and 122 (M-C₄H₈, 58); (Found M⁺ (EI), 178.09899. C₁₁H₁₄O₂ requires 178.09938).

6.12.2 (Z)-5-(4-*i*-butoxybenzylidene)thiazolidine-2,4-dione **177**.

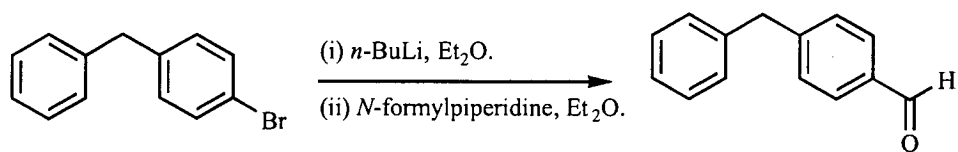
Thiazolidine-2,4-dione (475 mg, 4.06 mmol) and aldehyde **219** (724 mg, 4.06 mmol) were heated at reflux in toluene (27 cm³), containing piperidine (10 mg, 0.12 mmol) and acetic acid (12 mg, 0.20 mmol), under Dean and Stark conditions for 18 h. The solution was allowed to cool and the precipitate recovered by filtration. Recrystallisation from ethanol afforded the title compound as a yellow powder (723 mg, 2.61 mmol, 64 %); mp 176-178 °C (From EtOH); *R*_F (EtOAc-hexane, 1:1) 0.48; (Found : C, 60.70; H, 5.53; N, 5.00. C₁₄H₁₅NO₃S requires C, 60.63; H, 5.46; N, 5.05 %); ν_{\max} (KBr)/cm⁻¹ 3024 (olefinic C-H/Ph-H), 2953 (CH₂/CH₃), 1737 (C=O), 1687 (C=O), 1591 (Ph), 1568 (Ph), 1511 (Ph), 1467 (CH₂/CH₃), 1400 (CH₃) and 808 (Ph-H); δ_{H} (250 MHz; [²H₆] DMSO) 0.95 (6H, d, *J* 6.5, OCH₂CH(CH₃)₂), 2.00 (1H, tspt, *J* 6.5, OCH₂CH(CH₃)₂), 3.77 (2H, d, *J* 6.5, OCH₂CH(CH₃)₂), 7.03 (2H, d, *J* 9.0, Ph), 7.48 (2H, d, *J* 9.0, Ph), 7.69 (1H, s, olefinic CH) ppm; δ_{C} (63MHz; [²H₆] DMSO) 19.00 (CH₃), 27.72 (CH), 74.05 (CH₂), 115.36 (CH), 120.19 (qC), 125.42 (qC), 131.95 (CH), 132.14 (CH), 160.61 (qC), 167.47 (qC), 167.97 (qC) ppm; *m/z* (EI) 277 (M, 36 %), 221 (M-C₄H₈, 26), 206 (M-C(O)NHC(O), 10) and 150 (M-C₄H₈-C(O)NHC(O), 100); (Found M⁺ (EI), 277.07819. C₁₄H₁₅NO₃S requires 277.07727).

6.13 Synthesis Of (Z)-5-(4-benzylbenzylidene)thiazolidine-2,4-dione.

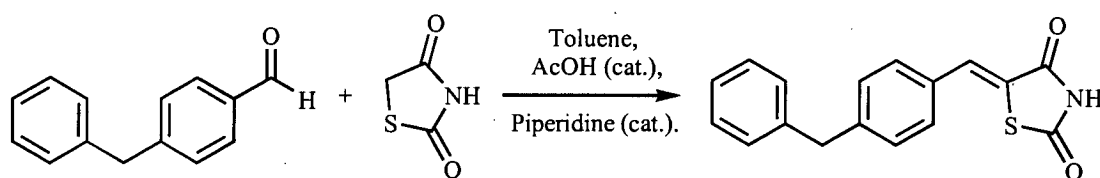
6.13.1 4-Benzylbromobenzene **221**.¹⁵¹



Sodium borohydride (2.28 g, 60.0 mmol) was added slowly to anhydrous TFA (50 cm³) which was cooled on ice under argon. This was warmed to ambient temperature and 4-bromobenzophenone **220** (2.61 g, 10.0 mmol) was added in anhydrous DCM (30 cm³). The resultant mixture was stirred at ambient temperature for a further 2 h before being cooled on ice and diluted with water (50 cm³). After neutralisation with sodium hydroxide pellets the mixture was extracted with diethyl ether (3 x 100 cm³). The organics were combined and washed successively with water (75 cm³), sat. aqueous sodium chloride (75 cm³) and water (75 cm³) before being dried over magnesium sulfate. The solvent was removed under reduced pressure and purification by column chromatography on silica gel, with ethyl acetate-hexane (1:10) as eluent, afforded the title compound as a clear oil (1.88 g, 7.59 mmol, 76 %); R_F (EtOAc-hexane, 1:10) 0.62; ν_{\max} (Film)/cm⁻¹ 3027 (Ph-H), 2910 (CH₂), 2844 (CH₂), 1603 (Ph), 1585 (Ph), 1486 (CH₂), 844 (Ph-H), 742 (Ph-H), 710 (C-Br) and 697 (Ph-H) (lit.,¹⁹³); δ_H (250 MHz; CDCl₃) 3.97 (2H, s, CH₂), 7.10 (2H, d, J 8.5, Ph), 7.22 (3H, m, Ph), 7.36 (2H, d, J 8.5, Ph), 7.45 (2H, d, J 8.5, Ph) ppm (lit.,¹⁹⁴); δ_C (63MHz; CDCl₃) 41.13 (CH₂), 119.78 (qC), 126.16 (CH), 128.43 (CH), 128.71 (CH), 130.51 (CH), 131.36 (CH), 139.94 (qC), 140.28 (qC) ppm (lit.,¹⁹⁵); m/z (EI) 246 (M(⁷⁹Br), 33 %) and 167 (M-Br, 100); (Found M^+ (EI), 246.00391. C₁₃H₁₁⁷⁹Br requires 246.00441).

6.13.2 4-Benzylbromobenzene **221**,¹⁵²

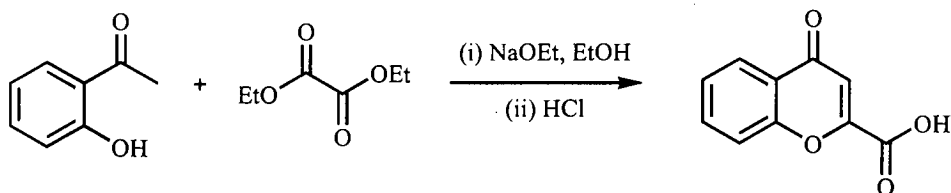
To a solution of 4-benzylbromobenzene **221** (1.50 g, 6.01 mmol) in anhydrous diethyl ether (6 cm³), under nitrogen, was added butyl lithium (4.3 cm³ of a 1.4 M solution in hexanes, 6.02 mmol). To this a solution of *N*-formylpiperidine (680 mg, 6.02 mmol) in anhydrous diethyl ether (15 cm³) was added over 2 min. The resultant solution was stirred for 15 min at ambient temperature, during which time a white precipitate had formed. The mixture was acidified to pH 2 with 3 M hydrochloric acid then extracted with diethyl ether (3 x 25 cm³). The organics were combined and washed successively with sat. aqueous sodium hydrogen carbonate (40 cm³), sat. aqueous sodium chloride (40 cm³) and water (40 cm³). The organics were dried over magnesium sulfate, filtered, and the solvent removed under reduced pressure to afford the title compound as a clear oil (1.18 g, 6.02 mmol, 100 %); *R*_F (EtOAc-hexane, 1:1) 0.70; ν_{max} (Film)/cm⁻¹ 3026 (Ph-H), 2967 (CH₂), 2848 (CH₂), 2717 (C(O)-H), 1694 (C=O), 1602 (Ph), 1574 (Ph), 1494 (CH₂), 734 (Ph-H), 722 (CH₂) and 698 (Ph-H); δ_{H} (250 MHz; CDCl₃) 4.07 (2H, s, CH₂), 7.29 (7H, m, Ph), 7.82 (2H, d, *J* 8.0, Ph), 9.97 (1H, s, C(O)H) ppm; δ_{C} (63MHz; CDCl₃) 41.84 (CH₂), 126.30 (CH), 128.47 (CH), 128.75 (CH), 129.33 (CH), 129.81 (CH), 134.42 (qC), 139.54 (qC) 148.20 (qC), 191.74 (qC) ppm; *m/z* (EI) 196 (M, 31 %) and 167 (M-C(O)H, 100); (Found *M*⁺ (EI), 196.08854. C₁₄H₁₂O requires 196.08882).

6.13.3 (Z)-5-(4-benzylbenzylidene)thiazolidine-2,4-dione **184**.

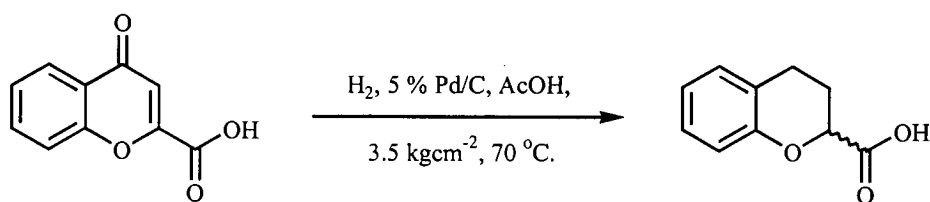
Thiazolidine-2,4-dione (537 mg, 4.59 mmol) and aldehyde **222** (900 mg, 4.59 mmol) were heated at reflux in toluene (52 cm³), containing piperidine (12 mg, 0.14 mmol) and acetic acid (8 mg, 0.14 mmol), under Dean and Stark conditions for 22 h.. The solution was allowed to cool before the solvent was removed under reduced pressure. Recrystallisation of the resultant solid from ethanol afforded the title compound as a yellow powder (2.85 g, 10.9 mmol, 88 %); mp 209-211 °C (From EtOH); *R*_F (EtOAc-hexane, 1:1) 0.50; (Found : C, 68.85; H, 4.44; N, 4.85. C₁₇H₁₃NO₂S requires C, 69.14; H, 4.44; N, 4.75 %); ν_{\max} (KBr)/cm⁻¹ 3020 (olefinic C-H/Ph-H), 2777 (CH₂), 1742 (C=O), 1690 (C=O), 1594 (Ph), 1508 (Ph), 1493 (CH₂), 837 (Ph-H), 746 (Ph-H), 723 (CH₂) and 685 (Ph-H); δ_{H} (250 MHz; [²H₆] DMSO) 3.98 (2H, s, CH₂), 7.24 (5H, m, Ph), 7.43 (2H, d, *J* 8.5, Ph), 7.51 (2H, d, *J* 8.5, Ph), 7.74 (1H, s, olefinic *H*) ppm; δ_{C} (63MHz; [²H₆] DMSO) 41.01 (CH₂), 122.83 (qC), 126.31 (CH), 128.68 (CH), 128.90 (CH), 129.76 (CH), 130.42 (CH), 130.97 (qC), 131.81 (CH), 145.67 (qC), 144.32 (qC), 167.50 (qC), 168.07 (qC) ppm; *m/z* (EI) 295 (M, 11 %) and 167 (M-C(O)NHC(O), 33); (Found M⁺ (EI), 295.06794. C₁₇H₁₃NO₂S requires 295.06670).

6.14 Synthesis Of (Z)-5-[(2*R*- and 2*S*-benzyl-3,4-dihydro-2*H*-1-benzopyran-6-yl)methylidene]thiazolidine-2,4-dione.

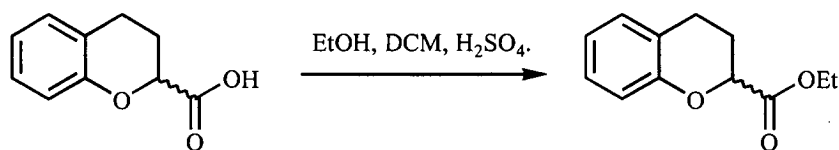
6.14.1 Chromone-2-carboxylic acid **225**.¹⁵³



A mixture of 2-hydroxyacetophenone **223** (66.4 g, 488 mmol) and diethyl oxalate **224** (107 g, 734 mmol) was added rapidly, with stirring, to a solution of sodium ethoxide (33.0 g, 486 mmol) in ethanol (230 cm³) and the mixture heated to 65 °C. After 30 min a yellow precipitate had formed and hydrochloric acid (300 cm³, s.g. 1.18) in water (350 cm³) was added and the solution heated under reflux for 4 h. After cooling overnight the pale brown precipitate was recovered by filtration and recrystallised from 70 % ethanol in water, and a second crop of crystals was obtained from the mother liquors, to afford the title compound as white crystals which were pure enough for use in the next stage (58.8 g, 309 mmol, 64 %); mp 250-251 °C dec. (From EtOH/H₂O) (lit.,¹⁵³ 250-251 °C dec.); *R_F* (EtOAc-hexane, 1:1) 0.16; ν_{max} (Film)/cm⁻¹ 3083 (olefinic C-H/Ph-H), 1733 (C=O), 1635 (C=O), 1588 (Ph), 1228 (C-O) and 749 (Ph-H); δ_{H} (250 MHz; [²H₆] DMSO) 6.88 (1H, s, olefinic *H*), 7.50 (1H, dd, *J* 8.0, Ph), 7.69 (1H, d, *J* 8.5, Ph), 7.85 (1H, dd, *J* 8.5, Ph), 8.02 (1H, d, *J* 8.0, Ph) ppm; δ_{C} (63MHz; [²H₆] DMSO) 113.62 (CH), 118.97 (CH), 123.84 (qC), 125.03 (CH), 126.14 (CH), 135.26 (CH), 153.32 (qC), 155.55 (qC), 161.51 (qC), 177.69 (qC) ppm; *m/z* (EI) 190 (M, 25 %) and 149 (M-C(O)CH, 100); (Found *M*⁺ (EI), 190.02671. C₁₀H₆O₄ requires 190.02661).

6.14.2 2,3-Dihydrobenzopyran-2-carboxylic acid **226**.¹⁵⁴

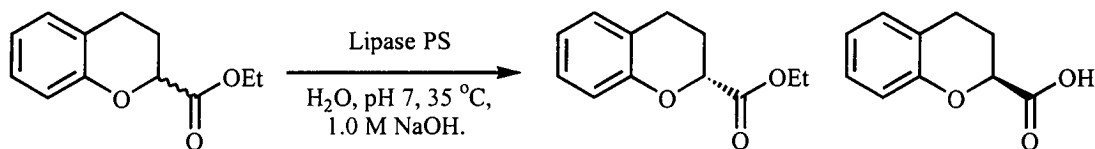
Acid **225** (69.0 g, 363 mmol) in acetic acid (400 cm³), containing 5 % Pd/C (7.50 g), was shaken at 70 °C under hydrogen at 3.5 kgcm⁻². The pressure was reapplied every time it dropped below 2.5 kgcm⁻². After the uptake of hydrogen had ceased the catalyst was removed by filtration through Celite™ which was washed with acetic acid (100 cm³). The filtrates were combined and the acetic acid removed under reduced pressure. The crude product was taken up in diethyl ether (500 cm³) and washed with sat. aqueous sodium hydrogen carbonate (2 x 125 cm³). The aqueous washings were combined and acidified to pH 1 with 2 M hydrochloric acid and extracted with diethyl ether (3 x 250 cm³). The organics were combined and dried over magnesium sulfate before the solvent was removed under reduced pressure to afford the title compound as white crystals, which were pure enough to be used in the next stage (36.2 g, 203 mmol, 56 %); mp 91-92 °C (lit.,¹⁵⁴ 93-96 °C); *R*_F (EtOAc-hexane, 1:1) 0.00; *ν*_{max} (Film)/cm⁻¹ 3041 (Ph-H), 2935 (CH₂), 1728 (C=O), 1612 (Ph), 1584 (Ph), 1488 (Ph), 1458 (CH₂), 1227 (C-O) and 753 (Ph-H); *δ*_H (250 MHz; [²H₆] DMSO) 2.11 (2H, m, CH₂), 2.66 (2H, m, CH₂), 4.76 (1H, dd, *J* 4.0 6.5, CHC(O)OH), 6.81 (1H, dd, *J* 7.0, Ph), 6.83 (1H, d, *J* 8.5, Ph), 7.05 (1H, d, *J* 7.0, Ph), 7.06 (1H, dd, *J* 8.5, Ph) ppm; *δ*_C (63MHz; [²H₆] DMSO) 22.63 (CH₂), 24.03 (CH₂), 72.94 (CH), 116.46 (CH), 120.40 (CH), 121.72 (qC), 127.44 (CH), 129.70 (CH), 153.55 (qC), 172.24 (qC) ppm; *m/z* (EI) 178 (M, 65 %), 133 (M-CO₂H, 100) and 105 (M-COCO₂H, 45); (Found *M*⁺ (EI), 178.06289. C₁₀H₁₀O₃ requires 178.06299).

6.14.3 2,3-Dihydrobenzopyran-2-carboxylic acid ethyl ester **227**.¹⁵⁵

Acid **226** (71.0 g, 399 mmol), ethanol (50.0 g, 1.23 mol) and 98 % sulfuric acid (1.5 cm³) were heated under reflux in DCM (175 cm³) for 23 h. The mixture was cooled to ambient temperature and diluted with water (300 cm³). The organic layer was separated and washed successively with sat. aqueous sodium hydrogen carbonate (250 cm³) and water (300 cm³) before being dried over magnesium sulfate and the solvent removed under reduced pressure. The crude product was purified by fractional distillation to afford the title compound as a colourless oil (74.2 g, 360 mmol, 90 %); bp 110-111 °C at 0.3 mmHg (lit.,¹⁵⁴ 116-117 °C at 0.5 mmHg); *R*_F (EtOAc-petroleum ether (bp 40-65 °C), 3:1) 0.89; *v*_{max} (Film)/cm⁻¹ 2979 (CH₂/CH₃), 2937 (CH₂/CH₃), 2850 (CH₂/CH₃), 1754 (C=O), 1601 (Ph), 1584 (Ph), 1458 (CH₂/CH₃), 1372 (CH₃), 1194 (C-O) and 755 (Ph-H); *δ*_H (250 MHz; CDCl₃) 1.28 (3H, t, *J* 7.0, OCH₂CH₃), 2.22 (2H, m, CH₂), 2.77 (2H, m, CH₂), 4.24 (2H, q, *J* 7.0, OCH₂CH₃), 4.70 (1H, dd, *J* 4.0 7.5, CHC(O)OEt), 6.85 (1H, dd, *J* 7.5, Ph), 6.89 (1H, d, *J* 8.0, Ph), 7.02 (1H, dd, *J* 7.5, Ph), 7.10 (1H, d, *J* 7.5, Ph) ppm (lit.,¹⁵⁵); *δ*_C (63MHz; CDCl₃) 14.03 (CH₃), 23.16 (CH₂), 24.47 (CH₂), 61.20 (CH₂), 73.59 (CH), 116.78 (CH), 120.61 (CH), 121.07 (qC), 127.37 (CH), 129.24 (CH), 153.34 (qC), 170.74 (qC) ppm; *m/z* (EI) 206 (M, 10 %), 133 (M-CO₂Et, 31) and 105 (M-COCO₂Et, 12); (Found *M*⁺ (EI), 206.09431. C₁₂H₁₄O₃ requires 206.09429).

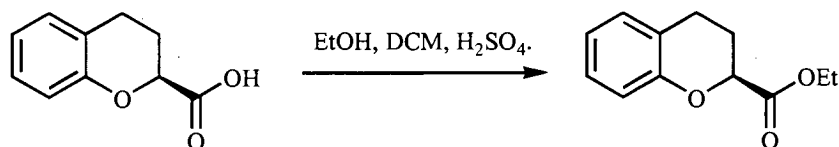
6.14.4 2,3-Dihydrobenzopyran-(2*R*) and (2*S*)-carboxylic acid ethyl ester, *R*- and *S*- 227.¹⁵⁵

2,3-Dihydrobenzopyran-(2*R*)-carboxylic acid ethyl ester, *R*-227:



Lipase PS was added to a mixture of racemic ester **227** (70.7 g, 343 mmol) in water (350 cm³) at 35 °C and pH 7.0. The reaction was allowed to proceed at this temperature with the pH being adjusted to 7.0 by addition of 1.0 M sodium hydroxide by means of a pH autotitrator. The reaction was stopped after the addition of 190.4 cm³ of the sodium hydroxide solution and the mixture allowed to cool to ambient temperature before being extracted with ethyl acetate (6 x 200 cm³). The combined organics were filtered through Celite™ then washed with water (3 x 200 cm³). The solvent was reduced to approximately one quarter of its volume and washed successively with 0.2 M sodium hydroxide (2 x 150 cm³), sat. aqueous sodium hydrogen carbonate (150 cm³) and water (150 cm³). The organics were dried over magnesium sulfate and the solvent removed under reduced pressure to afford the title compound as a clear oil (30.1 g, 146 mmol, 43 %, 97 % e.e.). Data coincided with that for the racemic ester except: $[\alpha]_D^{22}$ -8.8 ($c = 1.26$, MeOH) (lit.,¹⁵⁵ $[\alpha]_D$ -9.3 ($c = 1.24$, MeOH)); R_T (chiral gc) 52.29 min.

2,3-Dihydrobenzopyran-(2*S*)-carboxylic acid ethyl ester, *S*-227 :

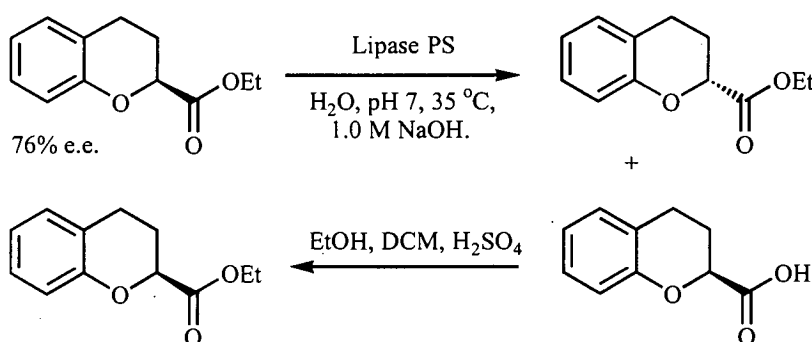


The aqueous fraction from the initial extraction was acidified to pH 1 with 2 M hydrochloric acid before extraction with ethyl acetate (6 x 200 cm³). The combined

organics were filtered through Celite™ and the solvent removed under reduced pressure to afford the residual acid as a white solid (22.4 g, 126 mmol, 37 %).

The acid (18.0 g, 101 mmol), ethanol (12.5 cm³) and 98 % sulfuric acid (0.5 cm³) were heated under reflux in DCM (150 cm³) for 22 h. The mixture was cooled to ambient temperature, dried over magnesium sulfate and the solvent removed under reduced pressure. Purification on silica gel, with ethyl acetate-petroleum ether (bp 40-65°) (3:1) as the eluent, afforded the product as a pale yellow oil (16.9 g, 82.0 mmol, 82 %, 76 % e.e.). Data coincided with that for the racemic ester except: $[\alpha]_D^{22} +6.3$ ($c = 1.20$, MeOH); R_T (chiral gc) 51.96 min.

6.14.5 Increasing the Enantiomeric Excess of 2,3-Dihydrobenzopyran-(2*S*)-carboxylic acid ethyl ester, *S*-227.

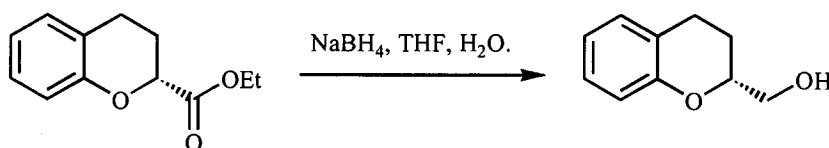


Lipase PS was added to a mixture of *S*-227 (15.5 g, 75.2 mmol) in water (160 cm³) at 35 °C and pH 7.0. The reaction was allowed to proceed at this temperature with the pH being adjusted to 7.0 by addition of 1.0 M sodium hydroxide by means of a pH autotitrator. The reaction was monitored by chiral gc and stopped after 20 h, at which point both ester enantiomers were present in equal amounts. The mixture was allowed to cool to ambient temperature before being filtered through a membrane and extracted with ethyl acetate (2 x 100 cm³). The aqueous layer was acidified to pH 1.0 with 2 M hydrochloric acid and extracted with ethyl acetate (3 x 100 cm³). The combined organics were dried over magnesium sulfate and the solvent removed under reduced pressure to afford the (*S*)-acid (5.63 g, 31.6 mmol, 41 %).

The acid (5.28 g, 29.7 mmol), ethanol (3.5 cm³) and 98 % sulfuric acid (0.3 cm³) were heated under reflux in DCM (45 cm³) for 21 h. The mixture was cooled to ambient temperature and washed successively with sat. aqueous sodium hydrogen carbonate (2 x 50 cm³), sat. aqueous sodium chloride (50 cm³) and water (50 cm³). The organics were dried over magnesium sulfate and the solvent removed under reduced pressure to afford the product as a pale yellow oil. (5.47 g, 26.6 mmol, 89 %, 84 % e.e.) Data coincided with that for the racemic ester except: $[\alpha]_{\text{D}}^{22} +7.1$ ($c = 1.23$, MeOH).

6.14.6 (2*R*)- and (2*S*)-(Hydroxymethyl)-2,3-dihydrobenzopyran, *R*- and *S*-228.¹⁵⁵

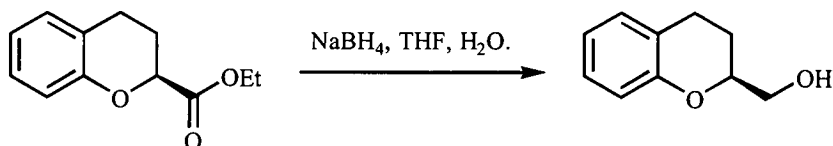
(2*R*)-(Hydroxymethyl)-2,3-dihydrobenzopyran, *R*-228 :



A solution of ester *R*-227 (29.0 g, 141 mmol) in THF (300 cm³) and water (30 cm³) was cooled on ice before sodium borohydride (12.5 g, 330 mmol) was added in portions, with stirring, over a 45 min period. This mixture was stirred for a further 13 h during which period it was allowed to slowly warm to ambient temperature. The mixture was again cooled on ice prior to the addition of acetone (28 cm³) which was followed by water (520 cm³) and DCM (21 cm³). The layers were separated and the aqueous layer was washed with DCM (3 x 200 cm³) and the combined organics were dried over magnesium sulfate before the solvent was removed under reduced pressure to afford the title compound as a clear oil (23.2 g, 141 mmol, 100 %); $[\alpha]_{\text{D}}^{22} -102.9$ ($c = 1.10$, MeOH) (lit.,¹⁵⁵ $[\alpha]_{\text{D}} -113.4$ ($c = 1.12$, MeOH)); R_{F} (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.26; ν_{max} (Film)/cm⁻¹ 3388 (O-H), 3040 (Ph-H), 2930 (CH₂), 2856 (CH₂), 1609 (Ph), 1583 (Ph), 1488 (CH₂), 1235 (C-O), 1046 (C-O) and 753 (Ph-H); δ_{H} (200 MHz; CDCl₃) 1.86 (2H, m, CH₂), 2.79 (3H, m, CH₂ and CH₂OH), 3.80 (2H, m, CH₂OH), 4.09 (1H, m, CHCH₂OH), 6.89 (2H, m, Ph), 7.08

(2H, m, Ph) ppm (lit.,¹⁵⁵); δ_c (50MHz; $CDCl_3$) 23.45 (CH_2), 24.21 (CH_2), 65.14 (CH_2), 76.20 (CH), 116.41 (CH), 120.10 (CH), 121.67 (qC), 127.00 (CH), 129.28 (CH), 154.21 (qC) ppm; m/z (EI) 164 (M, 36 %), 133 (M- CH_2OH , 79) and 105 (M-CO CH_2OH , 43); (Found M^+ (EI), 164.08409. $C_{10}H_{12}O_2$ requires 164.08373).

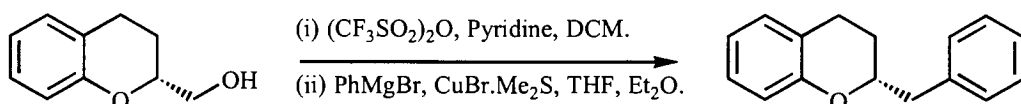
(2*S*)-(Hydroxymethyl)-2,3-dihydrobenzopyran, **S-228** :



The (*S*)-alcohol was prepared in a similar manner, on a 24 mmol scale from **S-227**, in 94 %. Data coincided with that for the (*R*)-isomer except: $[\alpha]_D^{22} +84.7$ ($c = 1.02$, MeOH).

6.14.7 (2*R*)- and (2*S*)-Benzyl-2,3-dihydrobenzopyran, *R*- and **S-229**.¹⁵⁵

(2*R*)-Benzyl-2,3-dihydrobenzopyran, **R-229** :

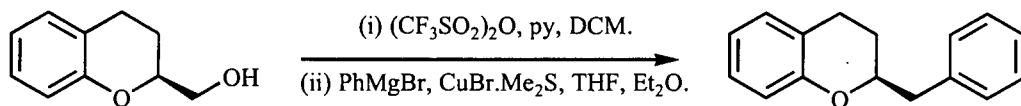


Alcohol **R-228** (4.00 g, 24.4 mmol) and pyridine (6.1 cm^3) in anhydrous DCM (69 cm^3) were cooled to $-15^\circ C$ under argon. A solution of trifluoromethanesulfonic anhydride (10.4 g, 30.1 mmol) in anhydrous DCM (29 cm^3) was slowly added dropwise and the mixture stirred at $-10^\circ C$ for 45 min. The reaction was quenched by the addition of DCM (100 cm^3) and 1 M hydrochloric acid (150 cm^3). The layers were separated and the organics were washed successively with water (150 cm^3), sat. aqueous sodium hydrogen carbonate (150 cm^3) and water (150 cm^3) before being dried over magnesium sulfate. The solvent was removed under reduced pressure,

ensuring that the temperature did not exceed 25 °C, to afford the triflate as a light orange oil which was used immediately in the following procedure.

Copper bromide dimethylsulfide (1.18 g, 5.70 mmol) was added to anhydrous THF (68 cm³) at -10 °C. Phenylmagnesium bromide (12.5 cm³ of a 3.0 M solution in diethyl ether, 37.5 mmol) was slowly added dropwise followed by a solution of the triflate in anhydrous THF (50 cm³). This mixture was stirred for a further 16 h, whilst being allowed to slowly warm to ambient temperature, before quenching with ethyl acetate (100 cm³) and sat. aqueous ammonium chloride (100 cm³). The layers were separated and the aqueous fraction was washed with ethyl acetate (100 cm³). The combined organics were washed successively with sat. aqueous ammonium chloride (100 cm³), water (100 cm³), sat. aqueous sodium hydrogen carbonate (100 cm³) and water (100 cm³) before being dried over magnesium sulfate. The solvent was removed under reduced pressure and the product purified by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:6) as eluent, to leave the title compound as a clear oil (4.79 g, 21.4 mmol, 87 %); $[\alpha]_D^{22}$ -98.7 (c = 1.02, MeOH) (lit.,¹⁵⁵ $[\alpha]_D$ -110 (c = 1, MeOH)); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:2) 0.69; ν_{\max} (Film)/cm⁻¹ 3063 (Ph-H), 3028 (Ph-H), 2928 (CH₂), 2862 (CH₂), 1608 (Ph), 1582 (Ph), 1488 (CH₂), 1236 (C-O), 1052 (C-O), 752 (Ph-H), 740 (Ph-H) and 699 (Ph-H); δ_H (200 MHz; CDCl₃) 1.84 (1H, m, CHH), 2.12 (1H, m, CHH), 2.87 (2H, m, CH₂), 3.02 (1H, dd, J 7.0 13.5, OCHCHHPh), 3.31 (1H, dd, J 6.0 13.5, OCHCHHPh), 4.37 (1H, m, OCHCH₂Ph), 7.02 (1H, d, J 8.0, Ph), 7.21 (2H, dd, J 8.0, Ph), 7.57 (5H, m, Ph) ppm (lit.,¹⁵⁵); δ_C (50MHz; CDCl₃) 24.36 (CH₂), 26.34 (CH₂), 41.60 (CH₂), 76.29 (CH), 116.61 (CH), 119.86 (CH), 121.72 (qC), 126.20 (CH), 127.00 (CH), 128.14 (CH), 129.29 (CH), 129.37 (CH), 137.66 (qC), 154.68 (qC) ppm; m/z (EI) 224 (M, 56 %), 133 (M-CH₂Ph, 100) and 105 (M-COCH₂Ph, 49); (Found M^+ (EI), 224.11991. C₁₆H₁₆O requires 224.12012).

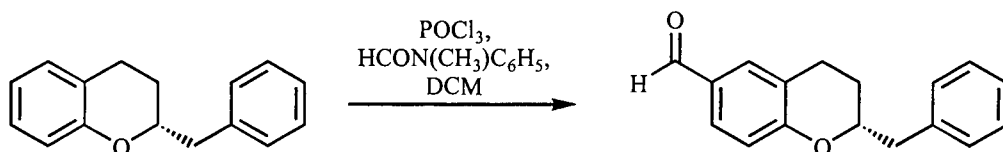
(2*S*)-Benzyl-2,3-dihydrobenzopyran, **S-229** :



The (*S*)-compound was prepared in a similar manner, on an 19 mmol scale from *S*-**228**, in 89 %. Data coincided with that for the (*R*)-isomer except: $[\alpha]_{\text{D}}^{22} +83.7$ ($c = 1.05$, MeOH).

6.14.8 (2*R*)- and (2*S*)-Benzyl-6-(formyl)-2,3-dihydro-4*H*-benzopyran, *R*- and *S*-230**.**¹⁵⁵

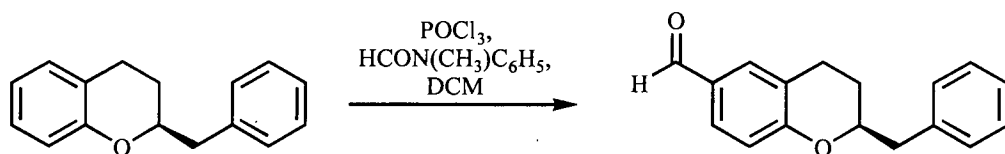
(2*R*)-Benzyl-6-(formyl)-2,3-dihydro-4*H*-benzopyran, **R-230** :



Phosphorous oxychloride (4.13 g, 27.1 mmol) and *N*-methylformanilide (3.68 g, 27.1 mmol) were stirred at ambient temperature for 5 min during which time the mixture solidified. **R-229** (3.50 g, 15.6 mmol) in DCM (4 cm³) was added and the mixture heated to 65 °C, with distillation of the DCM, before being heated at reflux for 16 h. The reaction was quenched by addition of DCM (100 cm³) and 15 % w/v aqueous sodium acetate (100 cm³). The layers were separated and the organics were washed successively with 15 % w/v aqueous sodium acetate (2 x 75 cm³), 1 M hydrochloric acid (75 cm³) and water (75 cm³). The organics were dried over magnesium sulfate and the solvent was removed under reduced pressure. Purified by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:3) as eluent, to leave the title compound as a low melting yellow solid (2.1 g, 8.3 mmol,

53 %); mp 65-66 °C (lit.,¹⁵⁵ 68-70 °C); $[\alpha]_D^{22}$ -152.3 ($c = 1.03$, MeOH) (lit.,¹⁵⁵ $[\alpha]_D$ -164.4 ($c = 1$, MeOH)); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:3) 0.59; ν_{\max} (Film)/ cm^{-1} 3058 (Ph-H), 3032 (Ph-H), 2930 (CH_2), 2850 (CH_2), 2734 ($\text{C}(\text{O})\text{-H}$), 1688 ($\text{C}=\text{O}$), 1604 (Ph), 1577 (Ph), 1493 (CH_2), 1266 (C-O), 740 (Ph-H), 716 (CH_2) and 704 (Ph-H); δ_H (250 MHz; CDCl_3) 1.71 (1H, m, CHH), 2.04 (1H, m, CHH), 2.81 (2H, m, CH_2), 2.91 (1H, dd, J 7.0 14.0, OCHCHHPh), 3.15 (1H, dd, J 6.0 14.0, OCHCHHPh), 4.30 (1H, m, OCHCH_2Ph), 6.90 (1H, d, J 8.5, Ph), 7.30 (5H, m, Ph); 7.61 (2H, m, Ph), 9.81 (1H, s, $\text{PhC}(\text{O})\text{H}$) ppm (lit.,¹⁵⁵); δ_C (63MHz; CDCl_3) 24.16 (CH_2), 25.85 (CH_2), 41.37 (CH_2), 77.30 (CH), 117.27 (CH), 122.26 (qC), 126.42 (CH), 128.24 (CH), 129.14 (qC), 129.32 (CH), 131.71 (CH), 137.01 (qC), 160.16 (qC), 190.75 (CH) ppm; m/z (EI) 252 (M, 37 %), 161 (M- CH_2Ph , 100), 133 (M-CO CH_2Ph , 8) and 105 (M-CO $\text{CH}_2\text{Ph-CO}$, 49); (Found M^+ (EI), 252.11528. $\text{C}_{17}\text{H}_{16}\text{O}_2$ requires 252.11503).

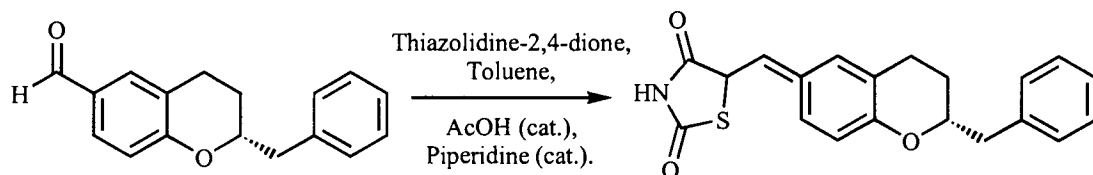
(2*S*)-Benzyl-6-(formyl)-2,3-dihydro-4*H*-benzopyran. **S-230** :



The (*S*)-aldehyde was prepared in a similar manner, on a 12 mmol scale from **S-229**, in 59 %. Data coincided with that for the (*R*)-isomer except: $[\alpha]_D^{22}$ +126.5 ($c = 1.01$, MeOH).

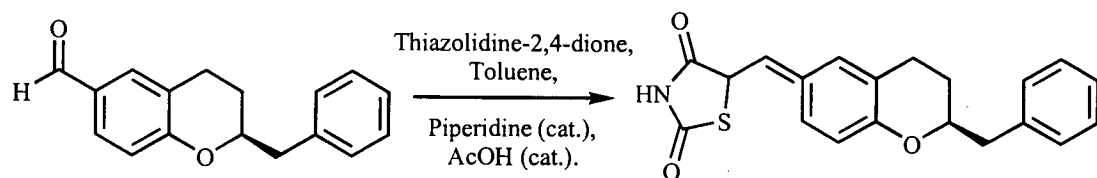
6.14.9 5-[(2*R*- and 2*S*-benzyl-3,4-dihydro-2*H*-1-benzopyran-6-yl)methylidene]thiazolidine-2,4-dione, *R*- and *S*-**231**.

5-[(2*R*-benzyl-3,4-dihydro-2*H*-1-benzopyran-6-yl)methylidene]thiazolidine-2,4-dione, *R*-**231** :



Thiazolidine-2,4-dione (761 mg, 6.50 mmol) and aldehyde *R*-**230** (1.64 g, 6.51 mmol) in toluene (50 cm³), containing piperidine (20 mg, 0.24 mmol) and acetic acid (20 mg, 0.33 mmol), were heated at reflux under Dean and Stark conditions for 24 h. The solvent was removed under reduced pressure and the product purified by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:1) as eluent, to leave the title compound as a yellow powder (2.14 g, 6.10 mmol, 94 %); mp 178-180 °C (lit.,⁵ 183-184 °C); $[\alpha]_D^{22}$ -135.6 (c = 1.02, DMSO); R_F (EtOAc-hexane, 1:1) 0.29; ν_{\max} (KBr)/cm⁻¹ 3026 (olefinic C-H/Ph-H), 2930 (CH₂), 2775 (CH₂), 1731 (C=O), 1688 (C=O), 1595 (Ph), 1570 (Ph), 1499 (Ph), 1255 (C-O), 812 (Ph-H), 751 (Ph-H) and 701 (Ph-H); δ_H (250 MHz; [²H₆] DMSO) 1.61 (1H, m, CHH), 1.96 (1H, m, CHH), 2.77 (2H, m, CH₂), 2.90 (1H, dd, J 6.5 14.0, OCHCHHPh), 3.03 (1H, dd, J 6.5 14.0, OCHCHHPh), 4.31 (1H, m, OCHCH₂Ph), 6.85 (1H, d, J 9.0, Ph), 7.27 (7H, m, Ph); 7.66 (1H, s, olefinic *H*) ppm; δ_C (63MHz; [²H₆] DMSO) 23.86 (CH₂), 25.81 (CH₂), 40.80 (CH), 76.99 (CH), 117.53 (CH), 119.92 (qC), 123.31 (qC), 125.05 (qC), 126.50 (CH), 128.41 (CH), 129.63 (CH), 132.19 (CH), 132.35 (CH), 137.68 (qC), 156.71 (qC), 167.61 (qC), 168.17 (qC) ppm; m/z (EI) 351 (M, 100 %), 280 (M-C(O)NHC(O), 32), 260 (M-CH₂Ph, 85) and 189 (M-C(O)NHC(O)-CH₂Ph, 33); (Found M^+ (EI), 351.09277. C₂₀H₁₇NO₃S requires 351.09292).

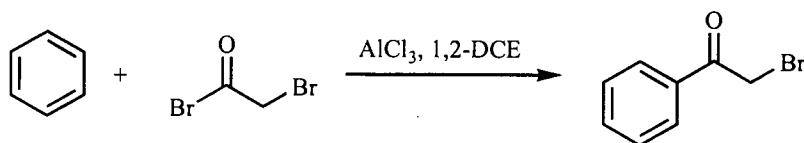
5-[(2*S*-benzyl-3,4-dihydro-2*H*-1-benzopyran-6-yl)methylidene]thiazolidine-2,4-dione, ***S*-231** :



The (*S*)-isomer was prepared in a similar manner, on a 6 mmol scale from ***S*-230**, in 86 %. Data coincided with that for the (*R*)-isomer except: $[\alpha]_D^{22} +100.8$ ($c = 0.96$, DMSO).

6.15 Synthesis of 5-[[5-benzoyl]methylidene]thiazolidine-2,4-dione.

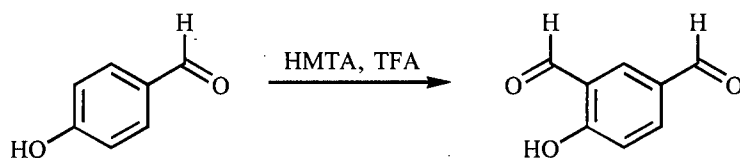
6.15.1 2-Bromoacetophenone **232**.¹⁵⁷



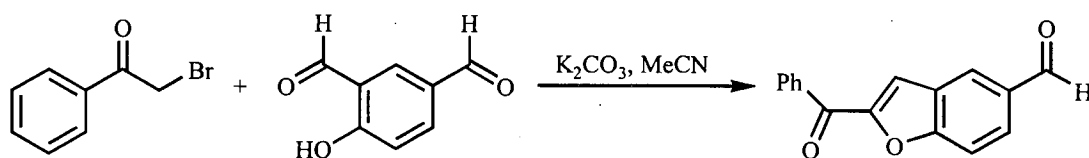
Benzene (1.58 g, 20.3 mmol) was added to a solution of aluminium chloride (2.57 g, 19.2 mmol) in 1,2-DCE (10 cm³) at 0 °C in one portion. Bromoacetyl bromide (3.41 g, 16.9 mmol) was added over 10 min, ensuring that the temperature did not exceed 10 °C. The mixture was warmed to ambient temperature and stirred for a further 3.5 h at which point water (17 cm³) was added slowly, ensuring that the temperature did not exceed 45 °C. The layers were separated and the organics were washed successively with 1 M hydrochloric acid (20 cm³) and water (20 cm³) before being dried over magnesium sulfate. The solvent was removed under reduced pressure to afford the title compound as a light brown powder (2.82 g, 14.2 mmol, 74 %); mp 49-51 °C (lit.,¹⁹⁶ 49-51 °C); R_F (EtOAc-hexane, 1:3) 0.52; ν_{max} (Film)/cm⁻¹ 3058 (Ph-H), 3002 (CH₂), 2954 (CH₂), 1694 (C=O), 1595 (Ph), 1580 (Ph), 1448 (CH₂) 746

(Ph-H), 704 (Ph-H) and 686 (C-Br) (lit.,¹⁹⁷); δ_{H} (250 MHz; CDCl_3) 4.45 (2H, s, CH_2Br), 7.48 (2H, t, J 7.5, Ph), 7.60 (1H, t, J 7.5, Ph), 7.97 (2H, d, J 7.5, Ph) ppm (lit.,¹⁹⁶); δ_{C} (63MHz; CDCl_3) 30.91 (CH_2), 128.67 (CH), 133.71 (CH), 133.78 (CH), 191.07 (qC) ppm; m/z (EI) 198 ($\text{M}^{(79)\text{Br}}$, 21 %), 119 (M-Br, 5), 105 (M- CH_2Br , 100) and 77 (M-C(O) CH_2Br , 19); (Found M^+ (EI), 197.96871. $\text{C}_8\text{H}_7\text{O}^{79}\text{Br}$ requires 197.96803).

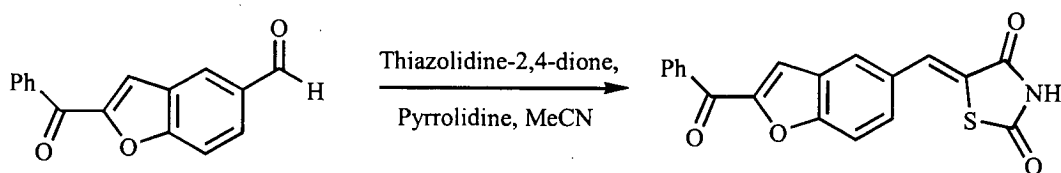
6.15.2 5-Formylsalicylaldehyde **233**.¹⁵⁷



HMTA (10.6 g, 76.0 mmol) was added, in one portion, to a solution of 4-hydroxybenzaldehyde (9.27 g, 76.0 mmol) in TFA (70 cm^3), under nitrogen. The resultant solution was heated at reflux for 24 h before being quenched with 3 M hydrochloric acid (120 cm^3) and allowed to cool before being extracted with DCM (4 x 145 cm^3). The organics were dried over magnesium sulfate and the solvent was removed under reduced pressure to leave a yellow oily solid. This was stirred on ice in ethanol (10 cm^3) and the solids were isolated by filtration and washed with cold ethanol (2 x 20 cm^3) to afford the title compound as a yellow solid (13.1 g, 65.7 mmol, 76 %); mp 110-111 $^{\circ}\text{C}$ (lit.,¹⁵⁷ 112-115 $^{\circ}\text{C}$); R_{F} (EtOAc) 0.65; ν_{max} (Film)/ cm^{-1} 3186 (O-H), 2837 (C(O)-H), 1689 (C=O), 1666 (C=O), 1571 (Ph) and 1156 (C-O) (lit.,¹⁵⁷); δ_{H} (250 MHz; [$^2\text{H}_6$] DMSO) 7.15 (1H, d, J 8.5, Ph), 7.99 (1H, dd, J 2.0 8.5, Ph), 8.18 (1H, d, J 2.0, Ph), 9.86 (1H, s, C(O)H), 9.98 (1H, s, C(O)H), 11.86 (1H br s, OH) ppm (lit.,¹⁵⁷); δ_{C} (63MHz, [$^2\text{H}_6$] DMSO) 118.34 (CH), 122.67 (qC), 128.48 (qC), 135.82 (CH), 137.45 (CH), 166.14 (CH), 190.15 (CH), 191.85 (CH) ppm (lit.,¹⁵⁷); m/z (EI) 150 (M, 61 %), 149 (M-H, 100), 121 (M-C(O)H, 19) and 93 (M-2C(O)H, 16); (Found M^+ (EI), 150.03193. $\text{C}_8\text{H}_6\text{O}_3$ requires 150.03169).

6.15.3 2-Benzoyl-5-benzofurancarboxaldehyde **234**.¹⁵⁷

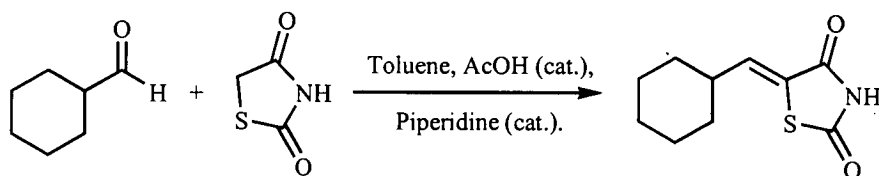
A mixture of 2-bromoacetophenone **232** (398 mg, 2.0 mmol), 5-formylsalicylaldehyde **233** (300 mg, 2.0 mmol) and potassium carbonate (276 mg, 4.6 mmol) were heated at reflux in acetonitrile (7.2 cm³) for 22 h. Water (14 cm³) was added and the mixture allowed to cool to ambient temperature. The precipitate was recovered by filtration and washed successively with water-acetonitrile (2:1) (2 x 2 cm³) and acetonitrile (2 x 2 cm³) before being dried under vacuum to afford the title compound as a pale yellow powder (280 mg, 1.12 mmol, 56 %); mp 155-156 °C (lit.,¹⁵⁷ 156.5-157 °C); *R_f* (EtOAc-hexane, 1:1) 0.55; ν_{\max} (Film)/cm⁻¹ 1695 (C=O), 1645 (C=O), 1610 (Ph), 1549 (Ph) and 1108 (C-O) (lit.,¹⁵⁷); δ_{H} (250 MHz; [²H₆] DMSO) 7.59 (4H, m, Ph), 7.73 (1H, d, *J* 8.5, Ph), 8.03 (3H, m, Ph), 8.25 (1H, d, *J* 1.0, Ph), 10.07 (1H, s, C(O)H) ppm (lit.,¹⁵⁷); δ_{C} (63MHz; [²H₆] DMSO) 113.24 (CH), 116.18 (CH), 126.78 (CH), 127.27 (qC), 128.53 (CH), 128.66 (CH), 129.29 (CH), 132.95 (qC), 133.18 (CH), 136.47 (qC), 153.51 (qC), 158.67 (qC), 183.74 (qC), 190.93 (CH) ppm (lit.,¹⁵⁷); *m/z* (EI) 250 (M, 21 %), 221 (M-C(O)H, 5), 173 (M-C₆H₅, 29) and 105 (M-C(O)C₈H₅O, 100).

6.15.4 (Z)-5-[[5-benzoyl]methylidene]thiazolidine-2,4-dione **235**.¹⁵⁷

Aldehyde **234** (200 mg, 0.80 mmol) was heated at reflux in acetonitrile (4.7 cm³) until dissolution had occurred. Pyrrolidine (6.8 μ l, 0.08 mmol) and thiazolidine-2,4-dione (112 mg, 0.96 mmol) were added and the mixture heated at reflux for 20 h. The precipitate was removed by filtration, washed with acetonitrile (2 cm³) and dried under vacuum at 90 °C to afford the title compound as a blood red powder (173 mg, 0.50 mmol, 63 %); mp 283-285 °C dec. (lit.,¹⁵⁷ 286-288 °C); R_F (EtOAc-hexane, 1:1) 0.50; ν_{\max} (KBr)/cm⁻¹ 3030 (olefinic C-H/Ph-H), 1740 (C=O), 1696 (C=O) 1647 (C=O), 1602 (Ph), 1551 (Ph) and 1126 (C-O) (lit.,¹⁵⁷); δ_H (600 MHz; [²H₆] DMSO) 7.61 (2H, m, Ph), 7.72 (1H, m, Ph), 7.76 (1H, m, Ph), 7.80 (1H, s, olefinic *H*), 7.85 (1H, s, olefinic *H*), 7.86 (1H, t, *J* 9.0, Ph), 8.00 (2H, m, Ph), 8.04 (1H, d, *J* 2.0, Ph) ppm (lit.,¹⁵⁷); δ_C (63MHz; [²H₆] DMSO) 113.32 (CH), 117.32 (CH), 125.23 (CH), 125.55 (qC), 127.81 (qC), 128.92 (CH), 129.35 (CH), 129.98 (CH), 130.89 (CH), 133.40 (CH), 136.67 (qC), 152.45 (qC), 155.59 (qC), 169.42 (qC), 170.25 (qC), 183.45 (qC) ppm (lit.,¹⁵⁷); m/z (EI) 349 (M⁺).

6.16 Knoevenagel Condensation Between Cyclohexane Carboxaldehyde and Thiazolidine-2,4-dione.

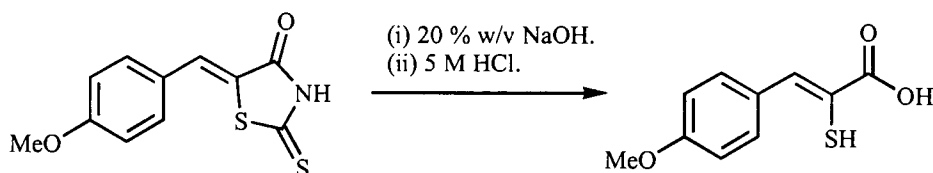
6.16.1 (Z)-5-cyclohexanemethylidene thiazolidine-2,4-dione **236**.



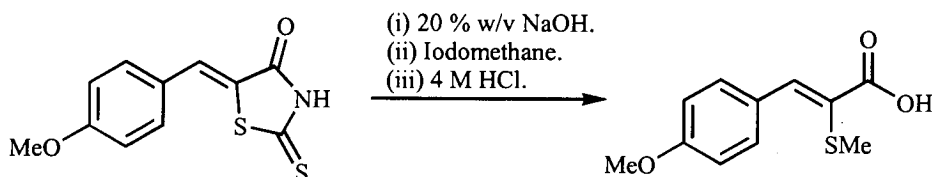
Thiazolidine-2,4-dione (1.76 g, 15.0 mmol) and cyclohexane carboxaldehyde (1.68 g, 15.0 mmol) were heated at reflux in toluene (100 cm³), containing acetic acid (27 mg, 0.45 mmol) and piperidine (38 mg, 0.45 mmol), under Dean and Stark conditions for 24h. The reaction mixture was cooled to ambient temperature and the solvent removed under reduced pressure. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:1) as eluent, afforded the title compound as a light brown powder (2.85 g, 13.5 mmol, 90 %); m.p. 140-141 °C; R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.58; ν_{\max} (KBr)/cm⁻¹ 3020 (olefinic C-H), 2930 (CH₂), 2850 (CH₂), 1741 (C=O), 1698 (C=O), 1628 (C=C), 1450 (CH₂) and 736 (CH₂); δ_H (600 MHz; [²H₆] DMSO) 1.22 (5H, m, cyclic CH), 1.63 (5H, d, cyclic CH), 2.05 (1H, m, cyclic CH), 6.74 (1H, d, J 9.5, olefinic H) ppm; δ_C (63MHz; [²H₆] DMSO) 24.87 (CH₂), 25.28 (CH₂), 30.61 (CH₂), 40.82 (CH), 125.17 (qC), 141.35 (CH), 166.48 (qC), 167.81 (qC) ppm; m/z (EI) 211 (M, 39 %), 168 (M-C(O)NH, 9) and 140 (M-C(O)NHC(O), 2); (Found M^+ (EI), 211.06745. C₁₀H₁₃NO₂S requires 211.06670).

6.17 Synthesis Of (Z)-2-sulfanyl-3-(4-methoxyphenyl)propenoic acid Derivatives.

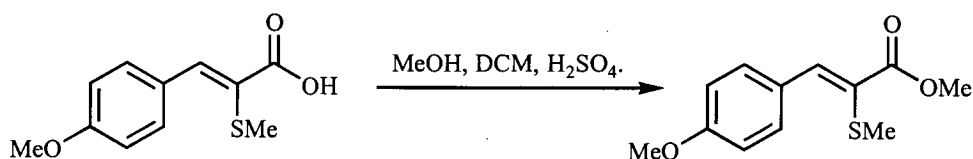
6.17.1 (Z)-2-sulfanyl-3-(4-methoxyphenyl)propenoic acid **237**.¹⁶¹



196 (4.05 g, 16.1 mmol) in 20 % w/v aqueous sodium hydroxide (27 cm³) was heated to 90 °C. The mixture was allowed to cool to ambient temperature before being acidified to pH 1 with 5 M hydrochloric acid. The precipitate was recovered by filtration and washed with methanol to afford the title compound as a yellow powder (3.17 g, 15.1 mmol, 94 %); mp 175-178 °C (lit.,¹⁹⁸ 177-178 °C); *R_f* (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.67; ν_{\max} (KBr)/cm⁻¹ 3063 (olefinic C-H/Ph-H), 3025 (olefinic C-H/Ph-H), 2926 (CH₃), 2835 (CH₃), 2567 (S-H), 1657 (C=O), 1607 (Ph), 1589 (Ph), 1508 (Ph), 1426 (CH₂) and 828 (Ph-H); δ_{H} (250 MHz; [²H₆] DMSO) 3.80 (3H, s, OCH₃), 6.95 (2H, d, *J* 9.0, Ph), 7.80 (1H, s, olefinic *H*), 7.84 (2H, d, *J* 9.0, Ph) ppm; δ_{C} (63MHz; [²H₆] DMSO) 55.48 (CH₃), 114.04 (CH), 125.29 (qC), 126.46 (qC), 133.29 (CH), 145.11 (CH), 160.98 (qC), 166.74 (qC); *m/z* (EI) 210 (M, 46 %), 209 (M-H, 58), 192 (M-H-OH, 12) and 164 (M-H-CO₂H, 58); (Found *M*⁺ (EI), 210.03591. C₁₀H₁₀O₃S requires 210.03507).

6.17.2 (Z)-2-methylsulfanyl-3-(4-methoxyphenyl)propenoic acid **238**.¹⁶⁰

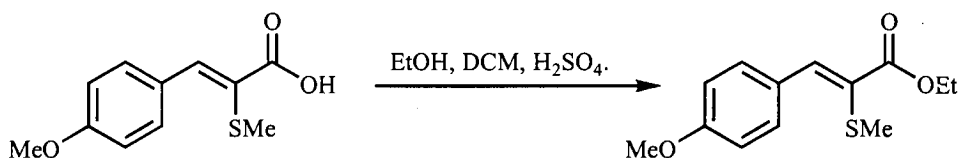
196 (1.50 g, 5.98 mmol) in 20 % w/v aqueous sodium hydroxide (10 cm³) was heated to 90 °C. Iodomethane (1.40 g, 9.86 mmol) was added and the mixture heated under reflux for 45 min at which point 4 M hydrochloric acid (5 cm³) was added and the solution allowed to cool to ambient temperature. The precipitate was recovered by filtration, washed with water (2 x 10 cm³) and recrystallised from methanol. A second crop of crystals was obtained from the mother liquors, to afford the title compound as a yellow powder (792 mg, 3.53 mmol, 59 %); mp 123-125 °C (From MeOH) (lit.,¹⁶⁰ 135 °C); R_F (EtOAc-hexane, 1:1) 0.11; ν_{\max} (KBr)/cm⁻¹ 3004 (olefinic C-H/Ph-H), 2916 (CH₃), 2841 (CH₃), 1670 (C=O), 1602 (Ph), 1578 (Ph), 1508 (Ph), 1419 (CH₃), 1355 (CH₃) and 826 (Ph-H); δ_H (250 MHz; CDCl₃) 2.35 (3H, s, SCH₃), 3.84 (3H, s, OCH₃), 6.93 (2H, d, J 9.0, Ph), 7.92 (2H, d, J 9.0, Ph), 8.03 (1H, s, olefinic H) ppm; δ_C (63MHz CDCl₃) 17.50 (CH₃), 55.18 (CH₃), 113.66 (CH), 123.66 (qC), 126.86 (qC), 133.23 (CH), 145.44 (CH), 160.96 (qC), 171.71 (qC); m/z (EI) 224 (M, 100 %) and 164 (M-CH₃-CO₂H, 17); (Found M^+ (EI), 224.05035. C₁₁H₁₂O₃S requires 224.05072).

6.17.3 (Z)- 2-methylsulfanyl-3-(4-methoxyphenyl)propenoic acid methyl ester **239**.

Acid **238** (1.03 g, 4.60 mmol), methanol (7.15 g, 223 mmol) and 98 % sulfuric acid (0.7 cm³) were heated under reflux in DCM (10 cm³) for 24 h. The solvent was removed under reduced pressure before ethyl acetate (100 cm³) was added. This was

washed successively with sat. aqueous sodium hydrogen carbonate (2 x 75 cm³), sat. aqueous sodium chloride (50 cm³) and water (50 cm³) before drying over magnesium sulfate. The solvent was removed under reduced pressure to afford the title compound as a yellow oil (980 mg, 4.12 mmol, 90 %); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.67; ν_{\max} (Film)/cm⁻¹ 3002 (olefinic C-H/Ph-H), 2951 (CH₃), 2929 (CH₃), 2838 (CH₃), 1712 (C=O), 1604 (Ph), 1566 (Ph), 1510 (Ph), 1434 (CH₃), 1352 (CH₃), and 831 (Ph-H); δ_H (250 MHz; CDCl₃) 2.27 (3H, s, SCH₃), 3.80 (3H, s, OCH₃), 3.83 (3H, s, OCH₃), 6.89 (2H, d, J 9.0, Ph), 7.81 (2H, d, J 9.0, Ph), 7.81 (1H, s, olefinic H) ppm; δ_C (63MHz; CDCl₃) 17.36 (CH₃), 52.41 (CH₃), 55.07 (CH₃), 113.45 (CH), 124.82 (qC), 127.01 (qC), 132.67 (CH), 142.58 (CH), 160.38 (qC), 166.57 (qC); m/z (EI) 238 (M, 84 %), 237 (M-H, 100), 207 (M-OMe, 4) and 179 (M-CO₂Me, 8); (Found M^+ (EI), 238.06550. C₁₂H₁₄O₃S requires 238.06637).

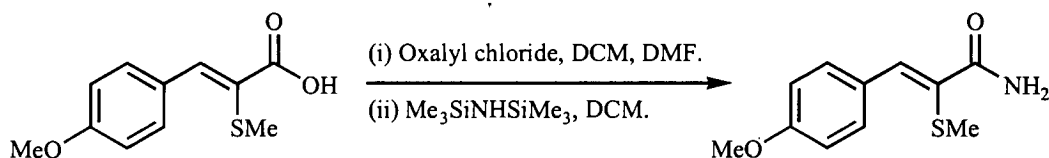
6.17.4 (Z)- 2-methylsulfanyl-3-(4-methoxyphenyl)propenoic acid ethyl ester **240**.



Acid **238** (990 mg, 4.42 mmol), ethanol (10.3 g, 223 mmol) and 98 % sulfuric acid (0.7 cm³) were heated under reflux in DCM (10 cm³) for 27 h. The solvent was removed under reduced pressure before ethyl acetate (100 cm³) was added. This was washed successively with sat. aqueous sodium hydrogen carbonate (2 x 75 cm³), sat. aqueous sodium chloride (50 cm³) and water (50 cm³) before drying over magnesium sulfate. The solvent was removed under reduced pressure to afford the title compound as a yellow oil (920 mg, 3.68 mmol, 83 %); R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.74; ν_{\max} (Film)/cm⁻¹ 3065 (olefinic C-H/Ph-H), 2979 (CH₂/CH₃), 2929 (CH₂/CH₃), 2838 (CH₂/CH₃), 1708 (C=O), 1604 (Ph), 1567 (Ph), 1510 (Ph), 1443 (CH₂/CH₃), 1365 (CH₃), 832 (Ph-H) and 759 (CH₂); δ_H (250 MHz; CDCl₃) 1.36 (3H, t, J 7.0, OCH₂CH₃), 2.29 (3H, s, SCH₃), 3.81 (3H, s, OCH₃), 4.31 (2H, q, J 7.0, OCH₂CH₃), 6.91 (2H, d, J 9.0, Ph), 7.80 (1H, s, olefinic H), 7.81 (2H,

d, J 8.5, Ph) ppm; δ_c (63MHz; CDCl_3) 14.00 (CH_3), 17.24 (CH_2), 54.97 (CH_3), 61.31 (CH_2), 113.32 (CH), 125.22 (qC), 127.01 (qC), 132.49 (CH), 141.95 (CH), 160.18 (qC), 165.94 (qC); m/z (EI) 252 (M, 81 %), 251 (M-H, 100), 207 (M-OEt, 15) and 179 (M-CO₂Et, 11); (Found M^+ (EI), 252.08206. $\text{C}_{13}\text{H}_{16}\text{O}_3\text{S}$ requires 252.08202).

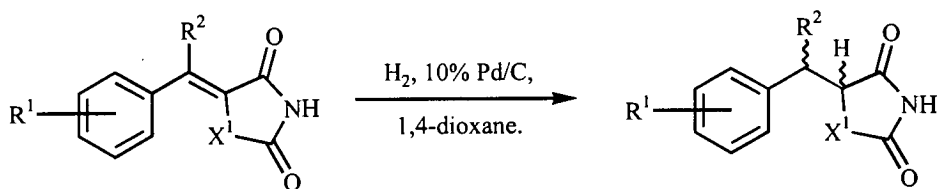
6.17.5 (Z)-2-methylsulfanyl-3-(4-methoxyphenyl)propenoic acid amide **241**.^{162,163}



Oxalyl chloride (685 mg, 5.39 mmol) was added to a solution of acid **238** (1.00 g, 4.46 mmol) in anhydrous DCM (10 cm^3) and DMF (1 drop) and the mixture heated to 25 °C. After 25 min the mixture was cooled to ambient temperature and the solvent was removed under reduced pressure to afford the crude acid chloride (1.33 g). 1,1,1,3,3,3-hexamethyldisilazane (1.77 g, 11.0 mol) in anhydrous DCM (28 cm^3) was added rapidly to a solution of the crude acid chloride (750 mg) in anhydrous DCM (10 cm^3) at 0 °C. The solution was allowed to warm to ambient temperature and was stirred for 48 h before being quenched with methanol (2 cm^3). This was washed successively with 5 % v/v sulfuric acid (2 x 10 cm^3) and sat. aqueous ammonium sulfate (2 x 10 cm^3) before drying over magnesium sulfate. The solvent was removed under reduced pressure to afford the title compound as a brown solid (537 mg, 2.41 mmol, 77 %); mp 209-211 °C; R_F (EtOAc-hexane, 1:1) 0.10; ν_{max} (Film)/ cm^{-1} 3357 (N-H), 3166 (N-H), 3002 (olefinic C-H/Ph-H), 2917 (CH_3), 2848 (CH_3), 1644 (Ph), 1604 (Ph), 1567 (Ph), 1509 (Ph), 1462 (CH_3), 1376 (CH_3) and 830 (Ph-H); δ_H (250 MHz; CDCl_3) 2.23 (3H, s, SCH_3), 3.82 (3H, s, OCH_3), 6.59 (2H, d, J 9.0, Ph), 7.89 (2H, d, J 9.0, Ph), 7.98 (1H, s, olefinic H) ppm; δ_c (63MHz; CDCl_3) 17.75 (CH_3), 55.14 (CH_3), 113.63 (CH), 124.83 (qC), 126.99 (qC), 132.62 (CH), 142.61 (CH), 160.53 (qC), 168.81 (qC); m/z (EI) 223 (M, 82 %), 222 (M-H, 91), 191

(M-OCH₃, 4) and 175 (M-OCH₃-NH₂, 100); (Found M⁺ (EI), 223.06585. C₁₁H₁₃NO₂S requires 223.06670).

6.18 Hydrogenation Of 5-Benzylidene 5-Membered Heterocycles.



6.18.1 General Procedures.

General Procedure 1 :

Unsaturated compound (0.44 mmol) in 1,4-dioxane (10 cm³) was hydrogenated over 10 % Pd/C (200 mg) until hplc indicated that conversion was complete. The catalyst was removed by filtration through Celite™ which was washed thoroughly with 1,4-dioxane before the solvent was removed under reduced pressure to afford crude product.

General Procedure 2 :

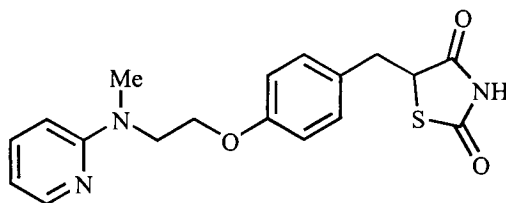
Unsaturated compound (0.44 mmol) in 1,4-dioxane (10 cm³) was hydrogenated over 10 % Pd/C (23 mg) until hplc indicated that conversion was complete. The catalyst was removed by filtration through Celite™ which was washed thoroughly with 1,4-dioxane before the solvent was removed under reduced pressure to afford crude product.

General Procedure 3 :

Unsaturated compound (0.91 mmol) in 1,4-dioxane (20 cm³) was hydrogenated over 10 % Pd/C (400 mg) until hplc indicated that conversion was complete. The catalyst was removed by filtration through Celite™ which was washed thoroughly with 1,4-dioxane before the solvent was removed under reduced pressure to afford crude product.

General Procedure 4 :

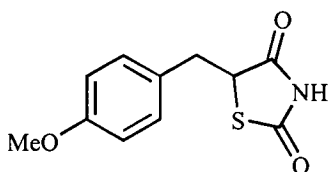
Unsaturated compound (0.72 mmol) in 1,4-dioxane (15 cm³) was hydrogenated over 10 % Pd/C (44 mg) until hplc indicated that conversion was complete. The catalyst was removed by filtration through Celite™ which was washed thoroughly with 1,4-dioxane before the solvent was removed under reduced pressure to afford crude product.

6.18.2 5-(4-{2-[methyl(2-pyridyl)amino]ethoxy}benzyl)thiazolidine-2,4-dione 2.

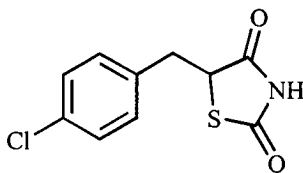
General procedure 3, for 48 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (2:1) as eluent, afforded the title compound as a white powder (210 mg, 0.59 mmol, 64 %); mp 149-151 °C (lit.,⁷ 153-155 °C); R_f (EtOAc-hexane, 2:1) 0.55; ν_{\max} (Film)/cm⁻¹ 2928 (CH₂/CH₃), 1752 (C=O), 1697 (C=O), 1603 (Ph), 1559 (Ph), 1510 (Ph), 1424 (CH₂/CH₃), 830 (Ph-H) and 735 (CH₂) (lit., ⁷); δ_H (250 MHz; [²H₆] DMSO) 3.04 (1H, dd, J 9.0 14.0, PhCHH), 3.06 (3H, s, NCH₃), 3.29 (1H, dd, J 4.5 14.0, PhCHH), 3.88 (2H, t, J 5.5, NCH₂), 4.10 (2H, t, J 5.5, OCH₂), 4.85 (1H, dd, J 4.5 9.0, PhCH₂CH), 6.57 (1H, m, Py), 6.63 (1H, d, J 8.5, Py), 6.87

(2H, d, J 8.5, Ph), 7.13 (2H, d, J 8.5, Ph), 7.49 (1H, m, Py), 8.07 (1H, m, Ph), 12.01 (1H, br s, NH) ppm (lit.,⁷); δ_c (63MHz; [2H_6] DMSO) 36.35 (CH₂), 37.15 (CH₃), 48.59 (CH₂), 53.13 (CH), 65.41 (CH₂), 105.84 (CH), 111.62 (CH), 114.39 (CH), 128.74 (qC), 130.49 (CH), 137.43 (CH), 147.64 (CH), 157.59 (qC), 158.12 (qC), 171.80 (qC), 175.81 (qC) ppm; m/z (EI) 357 (M, 6 %) and 121 (M-SC(O)NHC(O)CHCH₂-C₆H₄-OCH₂, 100); (Found M^+ (EI), 357.1151. C₁₈H₁₉N₃O₃S requires 357.1147).

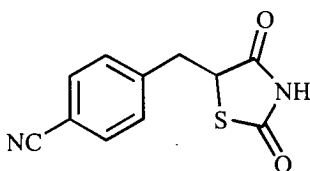
6.18.3 5-(4-methoxybenzyl)thiazolidine-2,4-dione **243**.



General procedure 1, for 24 h. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:1) as eluent, afforded the title compound as a white powder (79 mg, 0.33 mmol, 75 %); mp 109-110 °C; R_F (diethyl ether-hexane, 1:1) 0.42; ν_{max} (Film)/cm⁻¹ 3048 (Ph-H), 2905 (CH₂/CH₃), 1754 (C=O), 1693 (C=O), 1612 (Ph), 1513 (Ph), 1451 (CH₂/CH₃) and 1378 (CH₃); δ_H (250MHz; CDCl₃) 3.08 (1H, dd, J 9.5 14.0, PhCHH), 3.45 (1H, dd, J 4.0 14.0, PhCHH), 3.79 (3H, s, OCH₃), 4.49 (1H, dd, J 4.0 9.5, PhCH₂CH), 6.84 (2H, d, J 9.0, Ph), 7.14 (2H, d, J 9.0, Ph), 8.78 (1H, br s, NH) ppm; δ_c (63MHz; CDCl₃) 37.60 (CH₂), 53.66 (CH), 55.13 (CH₃), 114.08 (CH), 127.50 (qC), 130.18 (CH), 158.88 (qC), 170.61 (qC), 174.31 (qC) ppm; m/z (EI) 237 (M, 45 %), 121 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 237.04598. C₁₁H₁₁NO₃S requires 237.04597).

6.18.4 5-(4-chlorobenzyl)thiazolidine-2,4-dione 244.

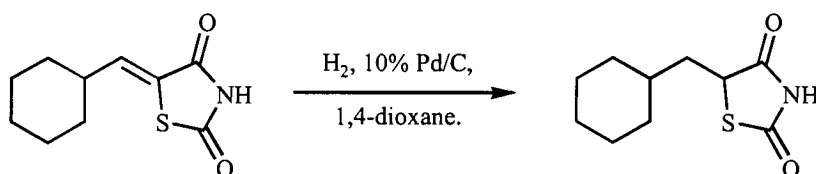
General procedure 1, for 24 h. Purification by column chromatography on silica gel, with diethyl ether-hexane (1:1) as eluent, afforded the title compound as a white solid (55 mg, 0.23 mmol, 53 %); mp 110-111 °C (lit.,² 110-111 °C); R_F (diethyl ether-hexane, 1:1) 0.40; ν_{\max} (Film)/cm⁻¹ 3064 (Ph-H), 1754 (C=O), 1694 (C=O), 1597 (Ph), 1511 (Ph) and 1492 (CH₂); δ_H (250MHz; CDCl₃) 3.12 (1H, dd, J 9.5 14.0, PhCHH), 3.46 (1H, dd, J 4.0 14.0, PhCHH), 4.51 (1H, dd, J 4.0 9.5, PhCH₂CH), 7.15 (2H, d, J 9.0, Ph), 7.29 (2H, d, J 9.0, Ph), 9.07 (1H, br s, NH) ppm; δ_C (63MHz, CDCl₃) 37.64 (CH₂), 52.94 (CH), 128.90 (CH), 130.48 (CH), 133.54 (qC), 133.89 (qC), 170.51 (qC), 174.28 (qC) ppm; m/z (EI) 241 (M(³⁵Cl), 20 %), 125 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 240.99698. C₁₀H₈NO₂S³⁵Cl requires 240.99643).

6.18.5 5-(4-cyanobenzyl)thiazolidine-2,4-dione 245.

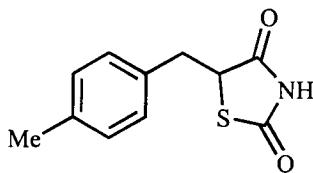
General procedure 3, for 24 h. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (40-65 °C) (1:1) as eluent, afforded the title compound as a white solid (60 mg, 0.26 mmol, 29 %); mp 182-183 °C; R_F (EtOAc-petroleum ether (bp 40-65 °C), 1:1) 0.20; ν_{\max} (KBr)/cm⁻¹ 3092 (Ph-H), 2846 (CH₂), 2239 (C≡N), 1755 (C=O), 1699 (C=O), 1605 (Ph), 1504 (Ph), 1448 (CH₂) and 841 (Ph-H); δ_H (250MHz; CDCl₃) 3.25 (1H, dd, J 9.0 14.0, PhCHH), 3.46 (1H, dd, J 5.0

14.0, PhCHH), 4.96 (1H, dd, J 5.0 9.0, PhCH₂CH), 7.45 (2H, d, J 9.0, Ph), 7.78 (2H, d, J 9.0, Ph) ppm; δ_c (63MHz, CDCl₃) 37.16 (CH₂), 51.96 (CH), 110.17 (qC), 118.91 (qC), 130.58 (CH), 132.45 (CH), 142.93 (qC), 171.60 (qC), 175.65 (qC) ppm; m/z (EI) 232 (M, 6 %), 116 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 232.03084. C₁₁H₈N₂O₂S requires 232.03065).

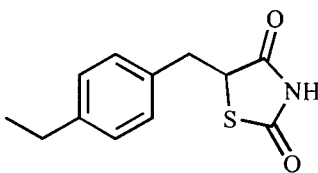
6.18.6 5-cyclohexanemethylenethiazolidine-2,4-dione 247.



General procedure 3, for 27 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a white solid (151 mg, 0.71 mmol, 78 %); m.p. 125-126 °C; R_F (EtOAc-hexane, 1:1) 0.63; ν_{\max} (Film)/cm⁻¹ 2918 (CH₂), 2838 (CH₂), 1749 (C=O) and 1688 (C=O); δ_H (600 MHz; CDCl₃) 0.91 (1H, m, cyclic CH), 0.99 (1H, m, cyclic CH), 1.19 (3H, m, cyclic CH), 1.38 (1H, m, cyclic CH), 1.68 (6H, m, SCHCHH and cyclic CH), 2.13 (1H, m, SCHCHH), 4.28 (1H, dd, J 4.0 11.0, SCHCH₂) ppm; δ_c (63MHz, CDCl₃) 25.66 (CH₂), 25.81 (CH₂), 26.02 (CH₂), 31.50 (CH₂), 33.47 (CH₂), 36.01 (CH), 40.53 (CH₂), 49.61 (CH), 171.45 (qC), 176.20 (qC) ppm; m/z (EI) 213 (M, 27 %) and 97 (M-SC(O)NHC(O)CH, 83); (Found M^+ (EI), 213.08295. C₁₀H₁₅NO₂S requires 213.08235).

6.18.7 5-(4-methylbenzyl)thiazolidine-2,4-dione 249.

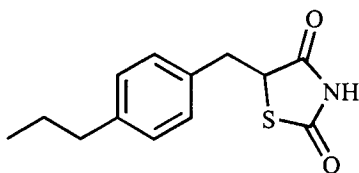
General procedure 1, for 24 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a white powder (98 mg, 0.44 mmol, 100 %); mp 73-75 °C; R_F (EtOAc-hexane, 1:1) 0.52; ν_{\max} (Film)/ cm^{-1} 2963 (CH_2/CH_3), 1754 ($\text{C}=\text{O}$), 1694 ($\text{C}=\text{O}$), 1516 (Ph), 1445 (CH_2/CH_3), 799 (Ph-H) and 720 (CH_2); δ_H (250MHz; CDCl_3) 2.32 (3H, s, CH_3), 3.07 (1H, dd, J 10.0 14.0, PhCHH), 3.50 (1H, dd, J 4.0 14.0, PhCHH), 4.51 (1H, dd, J 4.0 10.0, PhCH_2CH), 7.11 (4H, s, Ph), 9.20 (1H, br s, NH) ppm; δ_C (63MHz; CDCl_3) 20.92 (CH_3), 38.05 (CH_2), 53.52 (CH), 128.83 (CH), 129.38 (CH), 132.58 (qC), 137.18 (qC), 171.02 (qC), 174.70 (qC) ppm; m/z (EI) 221 (M, 45 %), 105 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 221.05190. $\text{C}_{11}\text{H}_{11}\text{NO}_2\text{S}$ requires 221.05105).

6.18.8 5-(4-ethylbenzyl)thiazolidine-2,4-dione 250.

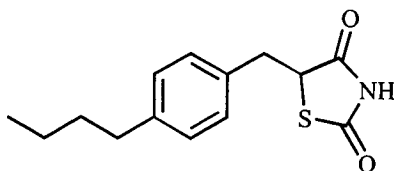
General procedure 3, for 30 h afforded the title compound as a white solid (146 mg, 0.62 mmol, 68 %); m.p. 102-104 °C; R_F (EtOAc-hexane, 1:1) 0.73; ν_{\max} (Film)/ cm^{-1} 3054 (Ph-H), 2965 (CH_2/CH_3), 2930 (CH_2/CH_3), 2872 (CH_2/CH_3), 1754 ($\text{C}=\text{O}$), 1695 ($\text{C}=\text{O}$), 1514 (Ph), 1438 (CH_2/CH_3), 825 (Ph-H), 737 (CH_2) and 702 (CH_2); δ_H (250MHz; CDCl_3) 1.22 (3H, t, J 7.5, CH_2CH_3), 2.63 (2H, q, J 7.5, CH_2CH_3), 3.08 (1H, dd, J 10.0 14.0, PhCHH), 3.52 (1H, dd, J 4.0 14.0, PhCHH), 4.52 (1H, dd, J 4.0

10.0, PhCH₂CH), 7.15 (4H, s, Ph), 9.10 (1H, br s, NH) ppm; δ_c (63MHz; CDCl₃) 15.29 (CH₃), 28.28 (CH₂), 38.15 (CH₂), 53.56 (CH), 128.18 (CH), 128.88 (CH), 132.89 (qC), 143.49 (qC), 171.01 (qC), 174.68 (qC) ppm; m/z (EI) 235 (M, 5 %), 119 (M-SC(O)NHC(O)CH, 100); (Found M⁺ (EI), 235.06683. C₁₂H₁₃NO₂S requires 235.06670).

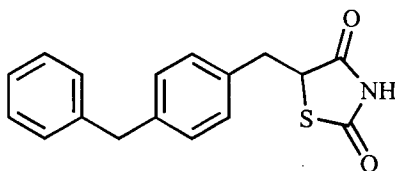
6.18.9 5-(4-propylbenzyl)thiazolidine-2,4-dione **251**.



General procedure 1, for 24 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a white solid (87 mg, 0.35 mmol, 79 %); mp 73-75 °C; R_f (EtOAc-hexane, 1:1) 0.57; ν_{max} (KBr)/cm⁻¹ 3054 (Ph-H), 2958 (CH₂/CH₃), 2930 (CH₂/CH₃), 2870 (CH₂/CH₃), 1754 (C=O), 1695 (C=O), 1514 (Ph), 1440 (CH₂/CH₃), 1328 (CH₃) and 790 (Ph-H); δ_H (200MHz; CDCl₃) 0.92 (3H, t, J 7.5, CH₂CH₂CH₃), 1.62 (2H, tq, J 7.5, CH₂CH₂CH₃), 2.56 (2H, t, J 7.5, CH₂CH₂CH₃), 3.08 (1H, dd, J 10.0 14.0, PhCHH), 3.52 (1H, dd, J 4.0 14.0, PhCHH), 4.51 (1H, dd, J 4.0 10.0, PhCH₂CH), 7.13 (4H, s, Ph), 8.30 (1H, br s, NH) ppm; δ_c (50MHz, CDCl₃) 13.69 (CH₃), 24.31 (CH₂), 37.50 (CH₂), 38.22 (CH₂), 53.54 (CH), 128.83 (CH), 132.86 (qC), 142.06 (qC), 170.61 (qC), 174.34 (qC) ppm; m/z (EI) 249 (M, 15 %), 133 (M-SC(O)NHC(O)CH, 100); (Found M⁺ (EI), 249.08228. C₁₃H₁₅NO₂S requires 249.08235).

6.18.10 5-(4-butylbenzyl)thiazolidine-2,4-dione 252.

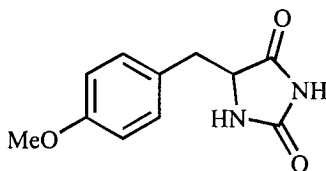
General procedure 1, for 24 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a white solid (88 mg, 0.33 mmol, 75 %); mp 75-77 °C; R_F (EtOAc-hexane, 1:1) 0.55; ν_{\max} (KBr)/ cm^{-1} 3053 (Ph-H), 2956 (CH_2/CH_3), 2928 (CH_2/CH_3), 2858 (CH_2/CH_3), 1754 (C=O), 1698 (C=O), 1514 (Ph), 1438 (CH_2/CH_3) and 1328 (CH_3); δ_H (250MHz; CDCl_3) 0.91 (3H, t, J 7.5, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.35 (2H, tq, J 7.5, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.58 (2H, tt, J 7.5, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2.58 (2H, t, J 7.5, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3.07 (1H, dd, J 10.0 14.0, PhCHH), 3.52 (1H, dd, J 4.0 14.0, PhCHH), 4.51 (1H, dd, J 4.0 10.0, PhCH_2CH), 7.13 (4H, s, Ph), 9.00 (1H, br s, NH) ppm; δ_C (63MHz, CDCl_3) 13.82 (CH_3), 22.23 (CH_2), 33.40 (CH_2), 35.16 (CH_2), 38.20 (CH_2), 53.58 (CH), 128.77 (CH) 128.83 (CH), 132.83 (qC), 142.27 (qC), 170.84 (qC), 174.53 (qC) ppm; m/z (EI) 263 (M, 8 %), 147 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 263.09726. $\text{C}_{14}\text{H}_{17}\text{NO}_2\text{S}$ requires 263.09800).

6.18.11 5-(4-benzylbenzyl)thiazolidine-2,4-dione 253.

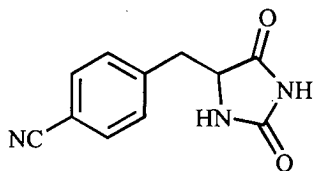
General procedure 1, for 24 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (2:3) as eluent, afforded the title compound as a white solid (75 mg, 0.25 mmol, 57 %); mp 114-116 °C; R_F (EtOAc-hexane, 2:3) 0.42; ν_{\max}

(KBr)/cm⁻¹ 3053 (Ph-H), 2908 (CH₂), 2842 (CH₂), 1754 (C=O), 1694 (C=O), 1513 (Ph), 1452 (CH₂), 734 (Ph-H) and 698 (Ph-H); δ_H (250MHz; CDCl₃) 3.08 (1H, dd, *J* 10.0 14.0, PhCHH), 3.52 (1H, dd, *J* 4.0 14.0, PhCHH), 3.97 (2H, s, PhCH₂Ph), 4.50 (1H, dd, *J* 4.0 10.0, PhCH₂CH), 7.24 (9H, m, Ph), 9.15 (1H, br s, NH) ppm; δ_C (63MHz, CDCl₃) 38.10 (CH₂), 41.33 (CH₂), 53.42 (CH), 126.02 (CH), 128.35 (CH), 128.76 (CH), 129.04 (CH), 129.21 (CH), 133.39 (qC), 140.47 (qC), 140.56 (qC), 170.96 (qC), 174.64 (qC) ppm; *m/z* (EI) 297 (M, 2 %), 181 (M-SC(O)NHC(O)CH, 12); (Found M⁺ (EI), 297.0930. C₁₇H₁₅NO₂S requires 297.0824).

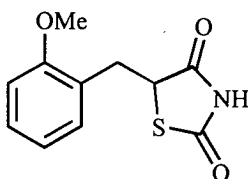
6.18.12 5-(4-methoxybenzyl)imidazolidine-2,4-dione **254**.



General procedure 2, for 18 h. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65°C) (3:1) as eluent, afforded the title compound as a white powder (87 mg, 0.40 mmol, 90 %); mp 177-179 °C (lit.,¹⁹⁹ 177-179 °C); *R_f* (EtOAc-petroleum ether (bp 40-65 °C), 3:1) 0.23; ν_{\max} (Film)/cm⁻¹ 1714 (C=O), 1611 (Ph), 1512 (Ph), 1419 (CH₂/CH₃) and 830 (Ph-H); δ_H (250MHz; [²H₆] DMSO) 2.86 (2H, d, *J* 5.0, PhCH₂), 3.71 (3H, s, OCH₃), 4.27 (1H, t, *J* 5.0, PhCH₂CH), 6.83 (2H, d, *J* 9.0, Ph), 7.09 (2H, d, *J* 9.0, Ph), 7.91 (1H, br s., NH), 10.40 (1H, br s., NH) ppm; δ_C (63MHz; [²H₆] DMSO) 35.53 (CH₂), 55.05 (CH₃), 58.67 (CH), 113.59 (CH), 127.37 (qC), 130.93 (CH), 157.27 (qC), 158.18 (qC), 175.37 (qC) ppm; *m/z* (EI) 220 (M, 75 %), 121 (M-NHC(O)NHC(O)CH, 85); (Found M⁺ (EI), 220.08435. C₁₁H₁₂N₂O₃ requires 220.08479).

6.18.13 5-(4-cyanobenzyl)imidazolidine-2,4-dione 255.

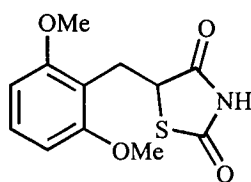
General procedure 4, for 24 h.. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (40-65°C) (3:1) as eluent, afforded the title compound as a white solid (70 mg, 0.33 mmol, 45 %); mp 208-209 °C; R_f (EtOAc-petroleum ether (bp 40-65 °C), 3:1) 0.29; ν_{\max} (Film)/cm⁻¹ 2237 (C≡N), 1708 (C=O) and 1594 (Ph); δ_H (200MHz; (CD₃)₂CO) 3.11 (1H, dd, J 6.5 14.0, PhCHH), 3.27 (1H, dd, J 4.5 14.0, PhCHH), 4.50 (1H, dd, J 4.5 6.5, PhCH₂CH), 7.08 (1H, br s, NH), 7.50 (2H, d, J 8.5, Ph), 7.72 (2H, d, J 8.5, Ph), 9.52 (1H, br s, NH) ppm; δ_C (50MHz; (CD₃)₂CO) 36.32 (CH₂), 57.91 (CH), 109.84 (qC), 115.19 (qC), 130.02 (CH), 131.16 (CH), 141.17 (qC), 155.59 (qC), 173.12 (qC) ppm; m/z (EI) 215 (M, 58 %), 116 (M-NHC(O)NHC(O)CH, 48); (Found M^+ (EI), 215.06953. C₁₁H₉N₃O₂ requires 215.06948).

6.18.14 5-(2-methoxybenzyl)thiazolidine-2,4-dione 256.

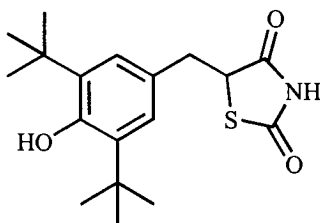
General procedure 3, for 46 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a white powder (178 mg, 0.75 mmol, 83 %); mp 105-107 °C; R_f (EtOAc-hexane, 1:1) 0.38; ν_{\max} (Film)/cm⁻¹ 3066 (Ph-H), 2940 (CH₂/CH₃), 2840 (CH₂/CH₃), 1752 (C=O), 1698 (C=O), 1602 (Ph), 1588 (Ph), 1494 (Ph), 1464 (CH₂/CH₃), 754 (Ph-H) and 720 (CH₂); δ_H (250MHz; CDCl₃) 2.92 (1H, dd, J 10.5 13.5, PhCHH), 3.76 (1H, dd, J 4.0

13.5, PhCHH), 3.84 (3H, s, OCH₃), 4.74 (1H, dd, *J* 4.0 10.5, PhCH₂CH), 6.89 (2H, m, Ph), 7.14 (1H, m, Ph), 7.27 (1H, m, Ph) ppm; δ_c (63MHz; CDCl₃) 34.30 (CH₂), 51.56 (CH), 55.09 (CH₃), 110.31 (CH), 120.49 (CH), 124.41 (qC), 128.97 (CH), 130.73 (CH), 157.33 (qC), 171.30 (qC) and 174.96 (qC) ppm; *m/z* (EI) 237 (M, 10 %), 121 (M-SC(O)NHC(O)CH, 93); (Found M⁺ (EI), 237.04555. C₁₁H₁₁NO₃S requires 237.04560).

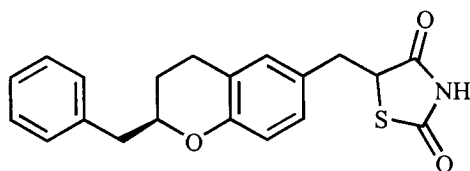
6.18.15 5-(2,6-dimethoxybenzyl)thiazolidine-2,4-dione 257.



General procedure 3, for 46 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (3:2) as eluent, afforded the title compound as a white powder (191 mg, 0.72 mmol, 79 %); mp 150-152 °C (lit.,²⁰⁰ 152-153 °C); *R_F* (EtOAc-hexane, 3:2) 0.48; ν_{\max} (Film)/cm⁻¹ 3053 (Ph-H), 2938 (CH₂/CH₃), 2837 (CH₂/CH₃), 1750 (C=O), 1699 (C=O), 1596 (Ph), 1588 (Ph), 1475 (CH₂/CH₃), 776 (Ph-H) and 721 (CH₂); δ_H (250MHz; CDCl₃) 3.33 (1H, dd, *J* 10.0 13.5, PhCHH), 3.50 (1H, dd, *J* 5.5 13.5, PhCHH), 3.81 (6H, s, 2OCH₃), 4.75 (1H, dd, *J* 5.5 10.5, PhCH₂CH), 6.54 (2H, d, *J* 8.5, Ph), 7.20 (1H, t, *J* 8.5, Ph), 8.68 (1H, br s, NH) ppm; δ_c (63MHz; CDCl₃) 26.51 (CH₂), 50.92 (CH), 55.53 (CH₃), 103.52 (CH), 112.65 (qC), 128.65 (CH), 158.36 (qC), 171.74 (qC) and 175.05 (qC) ppm; *m/z* (EI) 267 (M, 45 %), 151 (M-SC(O)NHC(O)CH, 100); (Found M⁺ (EI), 267.0571. C₁₂H₁₃NO₄S requires 2367.0565).

6.18.16 5-(3,5-di-*t*-butyl-4-hydroxybenzyl)thiazolidine-2,4-dione **258**.

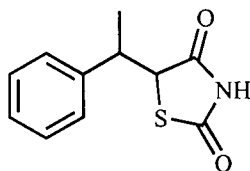
General procedure 3, for 48 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a white powder (264 mg, 0.79 mmol, 87 %); mp 72-74 °C; R_f (EtOAc-hexane, 1:1) 0.51; ν_{\max} (Film)/ cm^{-1} 3206 (O-H), 3064 (Ph-H), 2959 (CH_2/CH_3), 2915 (CH_2/CH_3), 2874 (CH_2/CH_3), 1754 (C=O), 1697 (C=O), 1591 (Ph), 1436 (CH_2/CH_3), 1392 (CH_3), 876 (Ph-H) and 737 (CH_2); δ_{H} (200MHz; CDCl_3) 1.42 (18H, s, 6 CH_3), 3.00 (1H, dd, J 10.5 14.0, PhCHH), 3.50 (1H, dd, J 3.5 14.0, PhCHH), 4.47 (1H, dd, J 3.6 10.5, PhCH $_2$ CH), 7.02 (2H, s, Ph) ppm; δ_{C} (50MHz; CDCl_3) 29.92 (CH_3), 34.17 (qC), 38.83 (CH_2), 54.31 (CH), 125.46 (CH), 126.54 (qC), 136.24 (qC), 153.08 (qC), 171.18 (qC), 174.90 (qC) ppm; m/z (EI) 335 (M, 10 %), 333 (M-2H, 51), 219 (M-SC(O)NHC(O)CH, 100).

6.18.17 5-[2*R*-benzyl-3,4-dihydro-2*H*-1-benzopyran-6-yl)methyl]thiazolidine-2,4-dione **259**.

General procedure 1, for 21 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a white powder (86 mg, 0.24 mmol, 56 %); mp 180-182 °C; R_f (EtOAc-hexane, 1:1) 0.43; ν_{\max} (Film)/ cm^{-1} 3062 (Ph-H), 3028 (Ph-H), 2923 (CH_2), 2850 (CH_2), 1753 (C=O),

1698 (C=O), 1614 (Ph), 1585 (Ph), 1498 (Ph), 1246 (C-O), 822 (Ph-H), 748 (Ph-H) and 701 (Ph-H); δ_{H} (250 MHz; CDCl_3) 1.69 (1H, m, CHH), 1.97 (1H, s, CHH), 2.73 (2H, m, CH_2), 2.86 (1H, dd, J 7.0 13.5, OCHCHHPh), 2.99 (1H, dd, J 10.0 14.0, SCHCHHPh), 3.13 (1H, dd, J 6.0 13.5, OCHCHHPh), 3.42 (1H, J 4.0 14.0, SCHCHHPh), 4.20 (1H, m, OCHCH₂Ph), 4.45 (1H, dd, J 4.0 10.0, SCHCH₂Ph), 6.75 (1H, d, J 8.0, Ph), 6.94 (2H, m, Ph); 7.28 (5H, m, Ph), 8.78 (1H, br s, NH) ppm; δ_{C} (63MHz; CDCl_3) 24.42 (CH_2), 26.22 (CH_2), 37.78 (CH_2), 41.59 (CH_2), 53.79 (CH), 76.56 (CH), 117.00 (CH), 122.18 (qC), 126.32 (CH), 127.05 (qC), 127.76 (CH), 128.24 (CH), 129.42 (CH), 130.09 (CH), 137.54 (qC), 154.21 (qC), 170.75 (qC), 174.36 (qC) ppm; m/z (EI) 353 (M, 37 %), 262 (M-CH₂Ph, 18) and 237 (M-SC(O)NHC(O)CH, 82); (Found M^+ (EI), 353.10962. $\text{C}_{20}\text{H}_{19}\text{NO}_3\text{S}$ requires 353.10857).

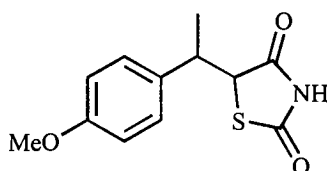
6.18.18 5-(1-methyl-1-phenylmethyl)thiazolidine-2,4-dione **260**.



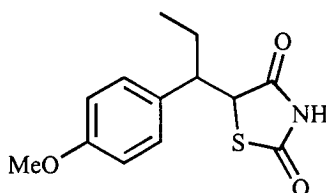
General procedure 3, for 27 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a white solid (187 mg, 0.85 mmol, 93 %); mp 86-88 °C; R_{F} (EtOAc-hexane, 1:1) 0.58; ν_{max} (Film)/ cm^{-1} 3061 (Ph-H), 2973 (CH_3), 1751 (C=O), 1694 (C=O), 1603 (Ph), 1495 (Ph), 1453 (CH_3), 1382 (CH_3), 764 (Ph-H) and 700 (Ph-H); δ_{H} (250MHz; CDCl_3) 1.41 (1.8H, d, J 7.0, CH_3), 1.48 (1.2H, d, J 7.0, CH_3), 3.73 (0.4H, m, PhCH(CH_3)CH), 3.81 (0.6H, m, PhCH(CH_3)CH), 4.54 (0.4H, d, J 5.0, PhCH(CH_3)CH), 4.60 (0.6H, d, J 3.5, PhCH(CH_3)CH), 7.25 (5H, m, Ph), 8.76 (0.4H, br s, NH), 9.20 (0.6H, br s, NH) ppm; δ_{C} (63MHz, CDCl_3) 13.36 (CH_3), 19.60 (CH_3), 39.92 (CH), 41.43 (CH), 58.58 (CH), 59.56 (CH), 126.97 (CH), 127.50 (CH), 127.68 (CH), 128.27 (CH), 128.80 (CH), 138.90 (qC), 141.32 (qC), 170.62 (qC), 171.26

(qC), 174.15 (qC), 174.39 (qC) ppm; m/z (EI) 221 (M, 4 %), 105 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 221.05136. $C_{11}H_{11}NO_2S$ requires 221.05105).

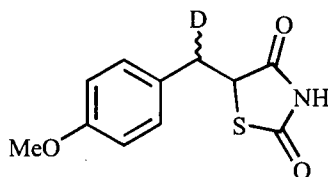
6.18.19 5-(1-methyl-1-(4-methoxyphenyl)methyl)thiazolidine-2,4-dione 261.



General procedure 3, for 27 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a gummy solid (183 mg, 0.73 mmol, 80 %); R_f (EtOAc-hexane, 1:1) 0.46; ν_{\max} (Film)/ cm^{-1} 3073 (Ph-H), 2956 (CH_3), 2912 (CH_3), 2824 (CH_3), 1752 (C=O), 1697 (C=O), 1610 (Ph), 1514 (Ph), 1458 (CH_3), 1380 (CH_3) and 832 (Ph-H); δ_H (250MHz; CDCl_3) 1.37 (1.9H, d, J 7.5, CH_3), 1.43 (1.1H, d, J 7.5, CH_3), 3.76 (1.2H, s, OCH_3), 3.77 (1H, m, $\text{PhCH}(\text{CH}_3)\text{CH}$), 3.78 (1.8H, s, OCH_3), 4.50 (0.35H, d, J 5.0, $\text{PhCH}(\text{CH}_3)\text{CH}$), 4.57 (0.65H, d, J 3.5, $\text{PhCH}(\text{CH}_3)\text{CH}$), 6.81 (0.7H, d, J 9.0, Ph), 6.86 (1.3H, d, J 9.0, Ph), 7.17 (2H, d, J 8.5, Ph), 8.91 (0.35H, br s, NH), 9.33 (0.65H, br s, NH) ppm; δ_C (63MHz, CDCl_3) 13.51 (CH_3), 19.71 (CH_3), 39.13 (CH), 40.53 (CH), 54.99 (CH_3), 55.09 (CH_3), 58.92 (CH), 59.86 (CH), 113.49 (CH), 114.04 (CH), 128.28 (CH), 129.35 (CH), 130.73 (qC), 133.32 (qC), 158.68 (qC), 158.78 (qC), 170.89 (qC), 171.46 (qC), 174.42 (qC), 174.53 (qC) ppm; m/z (EI) 251 (M, 14 %), 135 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 251.06160. $C_{12}H_{13}NO_3S$ requires 251.06162).

6.18.20 5-(1-ethyl-1-(4-methoxyphenyl)methyl)thiazolidine-2,4-dione 262.

General procedure 3, for 27 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (1:1) as eluent, afforded the title compound as a gummy solid (193 mg, 0.73 mmol, 80 %); R_f (EtOAc-hexane, 1:1) 0.51; ν_{\max} (Film)/ cm^{-1} 3074 (Ph-H), 2965 (CH_2/CH_3), 2934 (CH_2/CH_3), 2882 ($\text{CH}/\text{CH}_2/\text{CH}_3$), 2838 (CH_2/CH_3), 1752 ($\text{C}=\text{O}$), 1697 ($\text{C}=\text{O}$), 1611 (Ph), 1513 (Ph), 1459 (CH_2/CH_3) and 831 (Ph-H); δ_H (250MHz; CDCl_3) 0.82 (1.8H, t, J 7.5, CH_2CH_3), 0.87 (1.2H, t, J 7.5, CH_2CH_3), 1.68 (1.2H, m, CH_2CH_3), 2.05 (0.8H, m, CH_2CH_3), 3.77 (1.2H, s, OCH_3), 3.79 (1.8H, s, OCH_3), 4.50 (0.6H, d, J 3.5, $\text{PhCH}(\text{CH}_2\text{CH}_3)\text{CH}$), 4.57 (0.4H, d, J 5.0, $\text{PhCH}(\text{CH}_2\text{CH}_3)\text{CH}$), 6.81 (0.8H, d, J 9.0, Ph), 6.86 (1.2H, d, J 9.0, Ph), 7.15 (2H, d, J 8.5, Ph), 8.40 (0.4H, br s, NH), 8.85 (0.6H, br s, NH) ppm; δ_C (63MHz, CDCl_3) 11.63 (CH_3), 11.84 (CH_3), 22.19 (CH_2), 26.80 (CH_2), 47.54 (CH), 47.59 (CH), 55.03 (CH_3), 55.13 (CH_3), 57.41 (CH), 59.20 (CH), 113.54 (CH), 114.18 (CH), 128.47 (qC), 128.82 (CH), 130.10 (CH), 131.28 (qC), 158.86 (qC), 158.93 (qC), 170.41 (qC), 170.78 (qC), 174.12 (qC) ppm; m/z (EI) 265 (M, 6 %), 149 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 265.0765. $\text{C}_{13}\text{H}_{15}\text{NO}_3\text{S}$ requires 265.0773).

6.18.21 5-(4-methoxybenzyl-*d*)thiazolidine-2,4-dione 263.

General procedure 3, for 24 h. The product was dried at 40 °C under vacuum to afford the title compound as a white powder (169 mg, 0.71 mmol, 78 %); mp 104-105 °C; R_F (EtOAc-hexane, 1:1) 0.38; ν_{\max} (Film)/ cm^{-1} 3064 (Ph-H), 2936 (CH_3), 2838 (CH_3), 2788 (CH_3), 1754 ($\text{C}=\text{O}$), 1694 ($\text{C}=\text{O}$), 1610 (Ph), 1584 (Ph), 1514 (Ph), 1462 (CH_3) and 1383 (CH_3); δ_H (250MHz; CDCl_3) 3.07 (0.3H, d, J 9.5, PhCHD), 3.43 (0.7H, d, J 4.0, PhCDH), 3.79 (3H, s, OCH_3), 4.49 (~0.5H, d, J 4.0, PhCHDCH), 4.49 (~0.5H, d, J 9.5, PhCHDCH), 6.85 (2H, d, J 9.0, Ph), 7.14 (2H, d, J 9.0, Ph), 8.78 (1H, br s, NH) ppm; δ_C (63MHz; CDCl_3) 37.28 (t, J 20, CHD), 53.59 (CH), 55.12 (CH_3), 114.08 (CH), 127.45 (qC), 130.17 (CH), 158.89 (qC), 170.58 (qC), 174.29 (qC) ppm; m/z (EI) 238 (M, 15 %), 122 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 238.05210. $\text{C}_{11}\text{H}_{10}\text{DNO}_3\text{S}$ requires 238.05225).

7. Experimental : Biotransformations.

7.1 General Experimental.

Instrumentation and hplc conditions are shown in Sect. 6.1.

The *Rhodotorula rubra* CBS 6469 described in this work was obtained from the Centraalbureau Voor Schimmelcultures, Baarn, Netherlands.

The growth medium for *Rhodotorula rubra* CBS 6469 was prepared as follows :

Yeast extract (9 g) and mycological peptone (18 g) were dissolved in deionised water (900 cm³) and the pH adjusted to 7.0-7.2 with 2.0 M NaOH solution before a 30% w/v D-glucose solution (99 cm³) was added. This was autoclaved at 115 °C for 20 min.

The cells were grown and prepared for use as follows :

A 10 µl loopful of *Rhodotorula rubra* CBS 6469 was used to inoculate 180 cm³ of growth medium in a 500 cm³ baffled Erlenmeyer flask. This was shaken in an orbital shaker for 72 h at 30 °C and 220 rpm before a 2 cm³ aliquot was used to inoculate a similar flask which was shaken for 48 h at 30 °C and 220 rpm. The cells were recovered by centrifugation at 3000 rpm/4 °C for 10 min before being washed twice with water. Each time the cells were recovered by centrifugation at 3000 rpm/4 °C for 10 min. They were then washed with 0.1 M Tris-HCl buffer (pH 8.0), containing 5 % w/v sucrose, and recovered by centrifugation at 4500 rpm/4 °C for 15 min before being resuspended in the same buffer.

Low viscosity sodium alginate was purchased from Sigma (catalogue number A 2158) and the syringe needle used was supplied Aldrich (18 guage SS, 6 inches long, deflecting tip, catalogue number Z10271-7).

Mycological peptone and yeast extract were obtained from Oxoid.

7.2 Initial Optimisation Studies.

7.2.1 General Procedure.

Rhodotorula rubra CBS 6469 was grown in 180 cm³ of growth medium before being resuspended in 0.1 M Tris-HCl buffer (pH 8.0), containing 5% w/v sucrose, to a total volume of 84 cm³. Two 2 cm³ aliquots of the cell suspension were transferred to sample vials ensuring thorough vortexing between each transfer. 150 µl of a solution of **172** (0.33 mmol) in DMSO (6 cm³) was added to each aliquot and the mixture continuously shaken at 30 °C/ 220 rpm for 24 h.

7.2.2 Biotransformation Using Part of the Cell Suspension and 18.75 % v/v 1,4-Dioxane as Co-solvent.

The remaining 80 cm³ of cell suspension was diluted to 138 cm³ with the same buffer. 80 cm³ of the resulting cell suspension was added to a 500 cm³ Erlenmeyer flask. **172** (0.33 mmol) in 1,4-dioxane (15 cm³) was added and the mixture continuously shaken at 30 °C and 220 rpm for 24 h. At this point a 200 µl aliquot was removed and diluted with 800 µl of acetonitrile, filtered and subjected to hplc analysis. The extent of conversion was measured to be 25 %. Similar treatment of the 2 cm³ aliquots indicated 62 % conversion.

7.2.3 Biotransformation Using all of the Cell Suspension and 18.75 % v/v 1,4-Dioxane as Co-solvent.

The remaining 80 cm³ of cell suspension was added to a 500 cm³ Erlenmeyer flask. **172** (0.33 mmol) in 1,4-dioxane (15 cm³) was added and the mixture continuously shaken at 30 °C and 220 rpm for 24 h. At this point a 200 µl aliquot was removed

and diluted with 800 μl of acetonitrile, filtered and subjected to hplc analysis. The extent of conversion was measured to be 49 %. Similar treatment of the 2 cm^3 aliquots indicated 59 % conversion.

7.2.4 Biotransformation Using all of the Cell Suspension and 12 % v/v 1,4-Dioxane as Co-solvent.

The remaining 80 cm^3 of cell suspension was added to a 500 cm^3 Erlenmeyer flask. **172** (0.33 mmol) in 1,4-dioxane (9.6 cm^3) was added and the mixture continuously shaken at 30 $^{\circ}\text{C}$ and 220 rpm for 24 h. At this point a 200 μl aliquot was removed and diluted with 800 μl of acetonitrile, filtered and subjected to hplc analysis. The extent of conversion was measured to be 73 %. Similar treatment of the 2 cm^3 aliquots indicated 79 % conversion.

7.2.5 Biotransformation Using all of the Cell Suspension and 7.5 % v/v DMSO as Co-solvent.

The remaining 80 cm^3 of cell suspension was added to a 500 cm^3 Erlenmeyer flask. **172** (0.33 mmol) in DMSO (6 cm^3) was added and the mixture continuously shaken at 30 $^{\circ}\text{C}$ and 220 rpm for 24 h. At this point a 200 μl aliquot was removed and diluted with 800 μl of acetonitrile, filtered and subjected to hplc analysis. The extent of conversion was measured to be 58 %. Similar treatment of the 2 cm^3 aliquots indicated 57 % conversion.

7.3 Biotransformations Using Varying Amounts of Co-solvent.

7.3.1 General Procedure.

Rhodotorula rubra CBS 6469 was grown in 180 cm³ of growth medium before being resuspended in 0.1 M Tris-HCl buffer (pH 8.0), containing 5% w/v sucrose, to a total volume of 80 cm³. 2 cm³ aliquots of the cell suspension were transferred to sample vials ensuring thorough vortexing between each transfer. A series of solutions containing equal amounts of **172** in varying volumes of solvent were prepared by diluting a stock solution of **172**. An appropriate amount of each of these solutions, (equivalent to the addition of 1.6×10^{-5} mol of substrate) were added to the aliquots of cell suspension. These suspensions were continuously shaken at 30 °C and 220 rpm. At this point a 200 µl aliquot was removed and diluted with 800 µl of acetonitrile, filtered and subjected to hplc analysis to determine the extent of conversion.

7.3.2 Reduction of (Z)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione **172** with varying volumes of DMSO.

2 cm³ of a stock solution of **172** (531 mg, 2.26 mmol) in DMSO (14 cm³) was diluted with various volumes of DMSO. An appropriate volume of each of these solutions was added to aliquots of cell suspension and each aliquot was shaken at 30 °C and 220 rpm for 14 h. The results are shown in Table 7-1.

7.3.3 Reduction of (Z)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione **172 with varying volumes of 1,4-dioxane.**

1 cm³ of a stock solution of **172** (388 mg, 1.65 mmol) in 1,4-dioxane (10 cm³) was diluted with various volumes of 1,4-dioxane. An appropriate volume of each of these solutions was added to aliquots of cell suspension and each aliquot was shaken for 24 h. The results are shown in table 7-2.

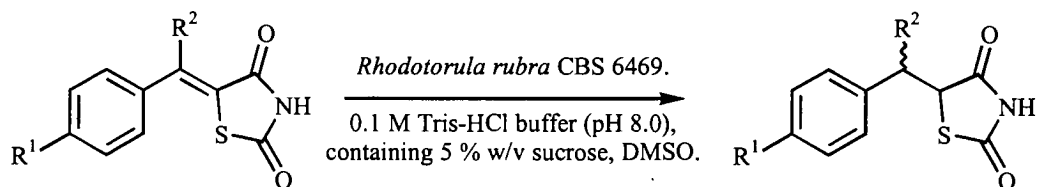
Solution	Volume of DMSO added (cm ³)	Volume of solution added (μl)	Ratio of DMSO to cell suspension (% v/v)	Conversion (%)
1	12	350	17.5	20
2	10	300	15.0	20
3	8	250	12.5	22
4	6	200	10.0	26
5	4	150	7.5	33
6	2	100	5.0	39
7	0	50	2.5	56

Table 7-1 : Relationship between rate of conversion and % v/v of DMSO.

Solution	Volume of 1,4-dioxane added (cm ³)	Volume of solution added (μl)	Ratio of 1,4-dioxane to cell suspension (% v/v)	Conversion (%)
1	20	1050	52.5	0
2	18	950	47.5	0
3	16	850	42.5	0
4	14	750	37.5	0
5	12	650	32.5	0
6	10	550	27.5	17
7	8	450	22.5	47
8	6	350	17.5	90

Table 7-2 : Relationship between rate of conversion and % v/v of 1,4-dioxane.

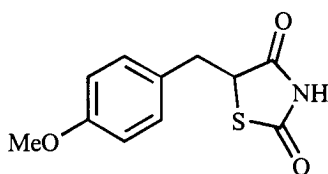
7.4 Biotransformations Using *Rhodotorula rubra* CBS 6469 to Afford Racemic Product.



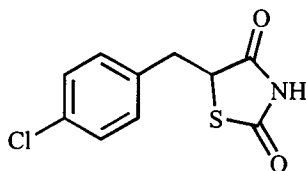
7.4.1 General procedure.⁷

Rhodotorula rubra CBS 6469 was grown in 180 cm³ of growth medium before being resuspended in 0.1 M Tris-HCl buffer (pH 8.0), containing 5% w/v sucrose, to a total volume of 80 cm³ in a 500 cm³ Erlenmeyer flask. Substrate (0.31-0.33 mmol in 6 cm³ DMSO) was added and the mixture continuously shaken at 30 °C and 220 rpm until hplc indicated complete conversion. The whole mixture, including cells, was continuously extracted into DCM (150 cm³) for 18 h. The solvent was dried over magnesium sulfate and removed under reduced pressure. In each case column chromatography on silica gel was performed to remove traces of DMSO.

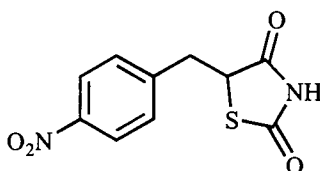
7.4.2 5-(4-methoxybenzyl)thiazolidine-2,4-dione **243**.



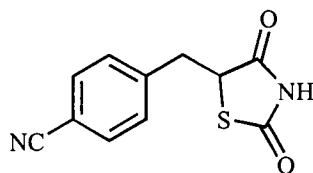
On 0.33mmol scale hplc indicated complete conversion to product after 58 h. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (2:1) as eluent, afforded the title compound as a white solid (68 mg, 0.29 mmol, 87 %). Data coincided with that for the same compound prepared by catalytic hydrogenation.

7.4.3 5-(4-chlorobenzyl)thiazolidine-2,4-dione 244.

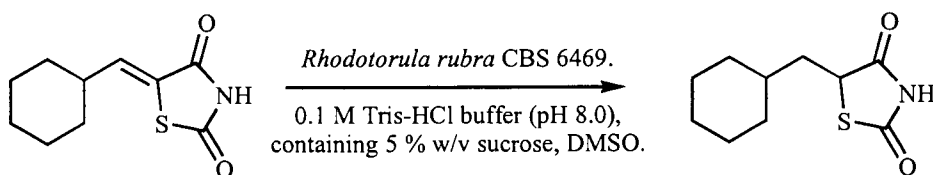
On 0.32 mmol scale hplc indicated complete conversion to product after 50h. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (2:1) as eluent, afforded the title compound as a white solid (32 mg, 0.13 mmol, 41 %). Data coincided with that for the same compound prepared by catalytic hydrogenation.

7.4.4 5-(4-nitrobenzyl)thiazolidine-2,4-dione 246.

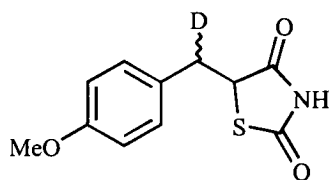
On 0.33 mmol scale hplc indicated complete conversion to product after 17 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (2:1) as eluent, afforded the title compound as a orange powder (68 mg, 0.27 mmol, 82 %); mp 176-178 °C; R_F (EtOAc-hexane, 3:2) 0.50; ν_{\max} (KBr)/ cm^{-1} 2924 (CH_2), 1758 (C=O), 1700 (C=O), 1596 (Ph), 1512 (NO_2), 1448 (CH_2), 1347 (NO_2), 841 (Ph-H) and 719 (CH_2); δ_H (250MHz; $(\text{CD}_3)_2\text{CO}$) 3.44 (1H, dd, J 9.0 14.0, PhCHH), 3.67 (1H, dd, J 5.0 14.0, PhCHH), 4.96 (1H, dd, J 5.0 9.0, PhCH₂CH), 7.64 (2H, d, J 9.0, Ph), 8.23 (2H, d, J 9.0, Ph), 10.76 (1H, br s, NH) ppm; δ_C (63MHz, $(\text{CD}_3)_2\text{CO}$) 36.46 (CH_2), 51.02 (CH), 122.60 (CH), 129.91 (CH), 143.86 (qC), 146.48 (qC), 169.24 (qC), 173.49 (qC) ppm; m/z (EI) 252 (M, 37 %), 220 (M-O₂, 7), 136 (M-SC(O)NHC(O)CH, 100); (Found M^+ (EI), 252.02074. $\text{C}_{10}\text{H}_8\text{N}_2\text{O}_4\text{S}$ requires 252.02048).

7.4.5 5-(4-cyanobenzyl)thiazolidine-2,4-dione **245**.

On 0.33 mmol scale hplc indicated complete conversion to product after 17 h. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (2:1) as eluent, afforded the title compound as a white solid (64 mg, 0.28 mmol, 84 %). Data coincided with that for the same compound prepared by catalytic hydrogenation.

7.4.6 5-(cyclohexylmethyl)thiazolidine-2,4-dione **247**.

On 0.33 mmol scale hplc indicated complete conversion to product after 24 h. Purification by column chromatography on silica gel, with ethyl acetate-hexane (3:2) as eluent, afforded the title compound as a white solid (58 mg, 0.27 mmol, 82 %). Data coincided with that for the same compound prepared by catalytic hydrogenation.

7.4.7 5-(4-methoxybenzyl-*d*)thiazolidine-2,4-dione **264**.

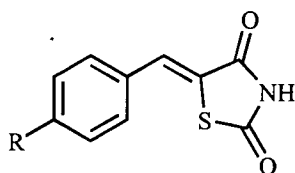
On 0.31 mmol scale hplc indicated complete conversion to product after 48 h. Purification by column chromatography on silica gel, with ethyl acetate-petroleum ether (bp 40-65 °C) (1:1) as eluent, afforded the title compound as a white solid (68 mg, 0.28 mmol, 90 %). Data coincided with that for the same compound prepared by catalytic hydrogenation **263** except: δ_{H} (250MHz; CDCl_3) 3.09 (0.5H, d, J 9.5, PhCHD), 3.43 (0.5H, d, J 3.5, PhCDH), 3.79 (3H, s, OCH_3), 4.49 (0.5H, d, J 3.5, PhCHDCH), 4.49 (0.5H, d, J 9.5, PhCHDCH), 6.85 (2H, d, J 9.0, Ph), 7.14 (2H, d, J 9.0, Ph), 8.37 (1H, br s, NH) ppm.

7.5 Screen of Compounds against *Rhodotorula rubra* CBS 6469.7.5.1 General Procedure.

Rhodotorula rubra CBS 6469 was grown in 180 cm³ of growth medium before being resuspended in 0.1 M Tris-HCl buffer (pH 8.0), containing 5% w/v sucrose, to a total volume of 80 cm³. 2 cm³ aliquots of the cell suspension were transferred to sample vials ensuring thorough vortexing between each transfer. To each aliquot 150 μl of a substrate solution (0.33 mmol in 6 cm³ DMSO) was added and the resultant mixture was incubated at 30 °C and 220 rpm for either 6 or 24 h. At this point a 200 μl aliquot was removed and diluted with 800 μl of acetonitrile, filtered and subjected to hplc analysis to determine the extent of conversion.

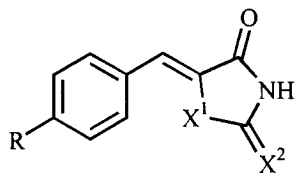
Every time a batch of compounds was screened two aliquots of cell suspension were used to biotransform **172** as a standard.

7.5.2 Screen Results : Reduction of (Z)-5-(4-susbtitutedbenzylidene)thiazolidine-2,4-diones.



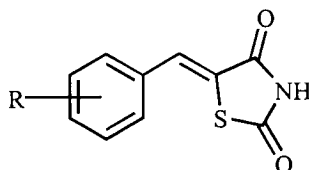
	R	Substrate	Reaction Time (h)	Conversion	Conversion of 172
170	H	yes	24	36	46
171	OH	yes	24	16	46
172	OMe	yes	24	46	46
173	OEt	yes	24	51	36
174	OPr	yes	24	32	46
175	OBu	yes	24	21	46
176	OPr ⁱ	yes	24	13	36
177	OBu ⁱ	yes	24	35	46
178	OBn	yes	24	6	46
179	SMe	yes	24	40	61
180	Me	yes	24	26	61
181	Et	no	24	----	----
182	Pr	no	24	----	----
183	Bu	no	24	----	----
184	Bn	no	24	----	----
185	F	yes	24	100	36
186	Cl	yes	24	88	36
187	Br	yes	24	84	74
188	I	yes	24	43	46
189	CN	yes	6	63	25
190	NO ₂	yes	6	44	25
191	OCF ₃	yes	6	38	25
248	OTBDMS	yes	24	93	46

7.5.3 Screen Results : Reduction of (Z)-5-(4-susbtitutedbenzylidene) 5-membered heterocycles.



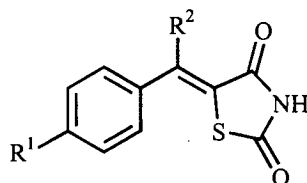
	R	X ¹	X ²	Substrate	Reaction Time (h)	Conversion	Conversion of 172
192	OMe	NH	O	no	24	-----	-----
193	Cl	NH	O	no	24	-----	-----
194	CN	NH	O	no	24	-----	-----
195	OMe	S	NH	n.d.	24	-----	-----
196	OMe	S	S	n.d.	24	-----	-----

7.5.4 Screen Results : Reduction of (Z)-5-(2-, 3-, di- and tri- substituted benzylidene)thiazolidine-2,4-diones.



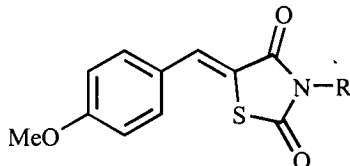
	R	Substrate	Reaction Time (h)	Conversion	Conversion of 172
197	2-OMe	n.d.	24	-----	-----
198	3-OMe	yes	24	15	43
199	2,6-OMe	n.d.	24	-----	-----
200	3,5-Bu ^t -4-OH	no	24	-----	-----

7.5.5 Screen Results : Reduction of (Z)-5-(1-alkyl-1-(4-substitutedphenyl)methylidene)thiazolidine-2,4-diones.



	R ¹	R ²	Substrate	Reaction Time (h)	Conversion	Conversion of 172
201	H	Me	no	24	----	----
202	OMe	Me	no	24	----	----
203	OMe	Et	no	24	----	----

7.5.6 Screen Results : Reduction of (Z)-5-(4-methoxybenzylidene)-3-substituted thiazolidine-2,4-diones.



	R	Substrate	Reaction Time (h)	Conversion	Conversion of 172
204	Me	yes	24	48	44
205	Bn	yes	24	49	44

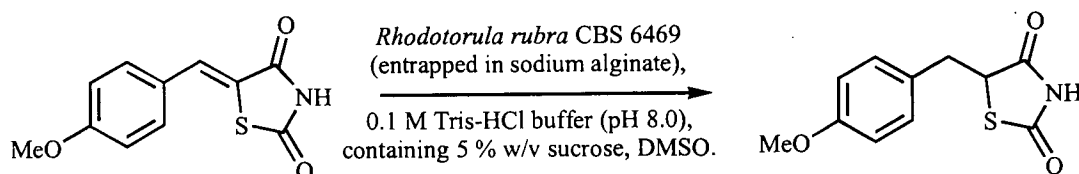
7.5.7 Screen Results : Reduction of Miscellaneous Thiazolidine-2,4-diones.

	Substrate	Reaction Time (h)	Conversion	Conversion of 172
<i>R</i> -231	no	24	-----	-----
<i>S</i> -231	no	24	-----	-----
235	yes	24	6 and 28	74
6	n.d.	24	-----	-----
236	yes	24	96	44

7.6 Immobilisation of *Rhodotorula rubra* CBS 6469.

7.6.1 Reduction of **172** by *Rhodotorula rubra* CBS 6469 Immobilised by Gel

Entrapment with Calcium Alginate.

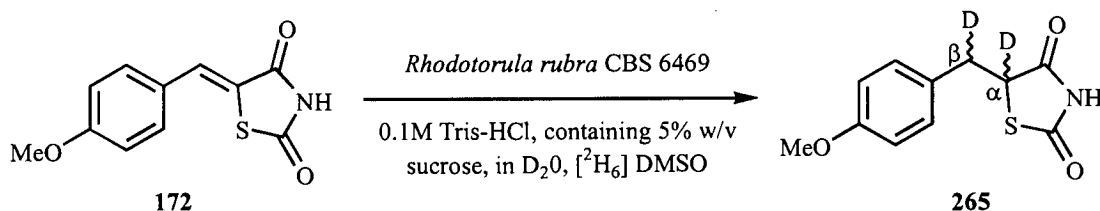


Rhodotorula rubra CBS 6469 was grown in 180 cm³ of growth medium before being resuspended in 0.1 M Tris-HCl buffer (pH 8.0), containing 5% w/v sucrose, to a total volume of 27 cm³. This suspension was mixed with a 2 % w/v solution of low viscosity sodium alginate solution in the same buffer (22 cm³)^a. This mixture was extruded from a syringe needle at a height of 8 cm into a 0.2 M aqueous solution of calcium chloride and allowed to harden for 30 min^b. The beads were recovered by filtration and washed with water (400 cm³) before being suspended in 0.1 M Tris-HCl buffer (pH 8.0), containing 5% w/v of sucrose, (80 cm³). **172** (75 mg, 0.32 mmol) was added in DMSO (6 cm³) and the mixture shaken at 30 °C and 220 rpm for 48 h at which point hplc had indicated complete conversion to product. The beads were removed by filtration and the filtrate was continuously extracted into DCM for 18 h. The DCM was dried over magnesium sulfate and evaporated under reduced pressure. Purification by column chromatography over silica gel, with ethyl acetate-hexane (3:2) as eluent, afforded the reduced compound as a white solid (75 mg, 0.19 mmol, 58 %). Data coincided with that of the same compound prepared by catalytic hydrogenation.

^a Low viscosity sodium alginate solution was shaken at 30 °C and 220 rpm for 24 h before use to ensure complete dissolution of the polymer.

^b The mixture was extruded at such a rate to ensure that the beads formed were uniform in size and spherical in appearance.

7.7 Mechanistic Studies.

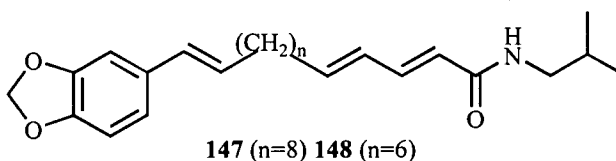
7.7.1 Biotransformation of (Z)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione **172** in Deuterated Medium.

Rhodotorula rubra CBS 6469 was grown in 180 cm³ of growth medium. After the initial wash with water the cells were resuspended in water, to a total volume of 100 cm³. 30 cm³ of this cell suspension was centrifuged at 3000 rpm/4 °C for 10 min before being washed with 0.1 M Tris-HCl buffer (pH 8.0), containing 5 % w/v sucrose, in D₂O (4 x 15 cm³). After each wash the cells were recovered by centrifugation at 4500 rpm/4 °C for 15 min. The cells were resuspended in the same buffer to a total volume of 20 cm³ before **172** (19 mg, 80 μmol) was added in [²H₆] DMSO (1.5 cm³) and shaken at 30 °C and 220 rpm. After 7 d the reaction appeared to have ceased at 87 % conversion and the whole mixture was continuously extracted into DCM (50 cm³) for 18 h. The DCM was dried over magnesium sulfate and evaporated under reduced pressure. Purification by column chromatography on silica gel, with ethyl acetate-hexane (3:2) as eluent, afforded a mixture of compounds **265** (20 mg) which was identified by ¹H nmr at 600 MHz, ²H nmr at 360 MHz and mass spectrometry.

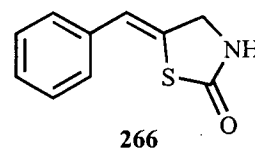
8. Future Work.

Given a longer time period to continue the research contained within this thesis there are certain points that would need to be addressed.

The possible reduction mechanism outlined in Fig. 4-10 indicates that the sulfur may be required to help activate the double bond. Utaka has shown that 2-heptenoic acid and its methyl ester are not reduced whereas the 2-chloro compound is.⁹⁰ Also, Das *et al.*, have shown that the isolated styryl double bonds of **147** and **148** are reduced but the enamide double bond is left untouched.¹⁰⁸



It may be possible that as the carbonyl at position 4 in the thiazolidine-2,4-dione ring is 'amide-like' this may not be a powerful enough activating group on its own. Incubation of a substrate from which the 4-carbonyl has been removed (eg. **266**) would help identify whether or not this functionality is required.



It would still be advantageous to develop a chiral hplc method to monitor the reduction of **172** and **215** at low pH, as one of the problems associated with these two reactions is the determination of when racemisation is occurring. Reduction of the deuterated substrate **215** to produce optically enriched product may help determine whether the hydrogen incorporated at the β position is in the *pro-R* or *pro-S* position, as well as the mode of addition across the double bond.

The relative reduction rates shown by this *Rhodotorula rubra* CBS 6469 system are specific to the conditions used. Identification and isolation of the responsible enzyme may allow the calculation of relative reduction rates with less contributing factors,

such as diffusion through the cell wall. If the protein is soluble this may also be possible with a crude cell free extract. The use of purified or partially purified enzyme preparations would allow the determination of the nature of the cofactor involved and investigation of cofactor recycling.

9. Appendix 1 : HPLC Data.

This appendix outlines the hplc retention times of unsaturated and saturated compounds as utilised to monitor hydrogenation reactions, and the screening of compounds against *Rhodotorula rubra* CBS 6469.

	Eluent Composition (%)		Retention Time (min)	
	Buffer ^c	Acetonitrile	Unsaturated Compound	Saturated Compound
6	70	30	26.38	25.72
170	80	20	13.87	11.57
171	90	10	28.38	12.82
172	80	20	35.12	23.77
173	80	20	53.42	32.15
174	70	30	15.38	12.89
175	70	30	28.59	22.80
176	70	30	13.63	12.11
177	70	30	28.25	23.40
178	70	30	27.11	24.23
179	80	20	50.72	34.27
180	80	20	56.93	46.38
181	70	30	17.42	15.28
182	60	40	8.40	11.43
183	60	40	20.92	12.55
184	60	40	6.50	4.00
185	80	20	17.18	13.83
186	80	20	46.35	31.57
187	80	20	50.92	35.60
188	80	20	65.98	42.35
189	80	20	14.67	10.72
190	80	20	23.27	14.07
191	70	30	25.85	14.68
192	80	20	23.52	10.38
193	80	20	42.18	-----
194	80	20	17.55	7.90
195	70	30	8.62	-----
196	80	20	39.70	-----

^c 50 mM NaH₂PO₄ (pH 7)

	Eluent Composition (%)		Retention Time (min)	
	Buffer	Acetonitrile	Unsaturated Compound	Saturated Compound
197	80	20	28.67	29.83
198	80	20	33.70	24.85
199	70	30	15.00	15.44
200	60	40	24.50	27.95
201	70	30	11.93	9.27 and 10.43
202	70	30	20.04	9.42 and 10.93
203	70	30	35.41	16.85 and 19.58
204	60	40	29.02	15.20
205	80	20	21.93	14.82
231	80	20	65.29	34.99
235	70	30	24.13	11.20 and 14.06
236	70	30	17.21	15.40
248	60	40	31.63	26.02

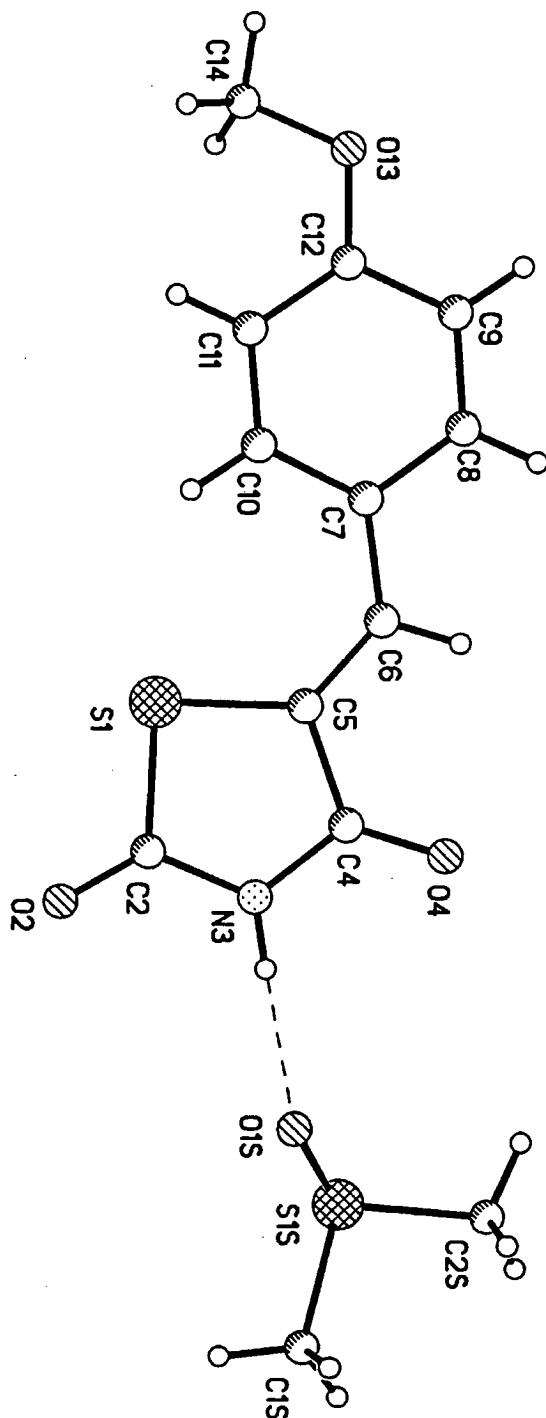
10. Appendix 2 : X-Ray Crystallography Data.**10.1 X-Ray Crystal Structure of 172.**

Fig. 10-1 : Representation of the Structure of 172 as its DMSO solvate.

A. CRYSTAL DATA

Empirical formula	$C_{13}H_{15}NO_4S_2$, $[C_{11}H_9NO_3S].DMSO$
Formula weight	313.38
Wavelength	1.54178 Å
Temperature	150(2) K
Crystal system	Monoclinic
Space group	P21/c
Unit cell dimensions	a = 16.372(4) Å alpha = 90 deg. b = 11.617(2) Å beta = 101.346(18) deg. c = 7.6659(17) Å gamma = 90 deg.
Volume	1429.5(5) Å ³
Number of reflections for cell	86 (20 < theta < 22 deg.)
Z	4
Density (calculated)	1.456 Mg/m ³
Absorption coefficient	3.500 mm ⁻¹
F(000)	656

B. DATA COLLECTION

Crystal description	Colourless block
Crystal size	0.54 x 0.42 x 0.27 mm
Theta range for data collection	2.75 to 69.97 deg.
Index ranges	-8 ≤ h ≤ 19, -14 ≤ k ≤ 11, -9 ≤ l ≤ 9
Reflections collected	3947
Independent reflections	2556 [R(int) = 0.0065]
Scan type	Omega-theta
Absorption correction	Optimised numerical (T _{min} = 0.183, T _{max} = 0.506)

C. SOLUTION AND REFINEMENT.

Solution	direct methods (SHELXS-97)
Refinement type	Full-matrix least-squares on F^2
Program used for refinement	SHELXL-97
Hydrogen atom placement	geometric
Hydrogen atom treatment	riding
Data / restraints / parameters	2556/0/185
Goodness-of-fit on F^2	1.066
Conventional R [$F > 4\sigma(F)$]	$R_1 = 0.0435$ [2405 data]
Weighted R (F^2 and all data)	$wR_2 = 0.1277$
Extinction coefficient	0.0034(6)
Final maximum delta/sigma	0.001
Weighting scheme calc $w^{-1} = [\sigma^2(F_o^2) + (0.0878P)^2 + 0.6934P]$ where $3P = (F_o^2 + 2F_c^2)$	
Largest diff. Peak and hole	0.413 and -0.561 e. \AA^{-3}

Table 10-1 : Crystal data and structure refinement for 172.

	x	y	z	U(eq)
S(1)	1516(1)	7862(1)	468(1)	33(1)
O(2)	3001(1)	7604(2)	-360(2)	46(1)
C(2)	2586(1)	8155(2)	475(3)	34(1)
N(3)	2850(1)	9077(2)	1545(2)	33(1)
O(4)	2451(1)	10349(2)	3491(2)	45(1)
C(4)	2284(1)	9573(2)	2435(3)	34(1)
C(5)	1453(1)	9028(2)	1883(3)	30(1)
C(6)	789(1)	9475(2)	2425(3)	33(1)
C(7)	-83(1)	9149(2)	2013(3)	32(1)
C(8)	-654(2)	9812(2)	2728(3)	36(1)
C(9)	-1496(2)	9593(2)	2354(3)	38(1)
C(10)	-409(1)	8239(2)	893(3)	37(1)
C(11)	-1254(2)	8009(2)	489(3)	37(1)
C(12)	-1803(1)	8697(2)	1202(3)	35(1)
O(13)	-2643(1)	8557(2)	861(2)	41(1)
C(14)	-2989(2)	7756(2)	-499(3)	40(1)
S(1S)	5012(1)	709(1)	2035(1)	35(1)
O(1S)	4500(1)	-279(2)	2493(3)	48(1)
C(1S)	6071(2)	286(2)	2715(4)	49(1)
C(2S)	5005(2)	1778(2)	3701(4)	46(1)

Table 10-2 : Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **172**. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

S(1)-C(5)	1.751(2)
S(1)-C(2)	1.783(2)
O(2)-C(2)	1.205(3)
C(2)-N(3)	1.367(3)
N(3)-C(4)	1.380(3)
O(4)-C(4)	1.207(3)
C(4)-C(5)	1.484(3)
C(5)-C(6)	1.343(3)
C(6)-C(7)	1.450(3)
C(7)-C(10)	1.400(3)
C(7)-C(8)	1.402(3)
C(8)-C(9)	1.376(4)
C(9)-C(12)	1.393(3)
C(10)-C(11)	1.383(3)
C(11)-C(12)	1.393(3)
C(12)-O(13)	1.358(3)
O(13)-C(14)	1.428(3)
S(1S)-O(1S)	1.5031(18)
S(1S)-C(1S)	1.779(3)
S(1S)-C(2S)	1.784(3)
C(5)-S(1)-C(2)	91.63(10)
O(2)-C(2)-N(3)	126.2(2)
O(2)-C(2)-S(1)	123.58(18)
N(3)-C(2)-S(1)	110.22(16)
C(2)-N(3)-C(4)	117.42(19)
O(4)-C(4)-N(3)	123.9(2)
O(4)-C(4)-C(5)	125.8(2)
N(3)-C(4)-C(5)	110.32(18)
C(6)-C(5)-C(4)	119.97(19)
C(6)-C(5)-S(1)	129.85(18)
C(4)-C(5)-S(1)	110.13(15)
C(5)-C(6)-C(7)	130.9(2)
C(10)-C(7)-C(8)	116.9(2)
C(10)-C(7)-C(6)	124.8(2)
C(8)-C(7)-C(6)	118.3(2)
C(9)-C(8)-C(7)	122.3(2)
C(8)-C(9)-C(12)	119.5(2)
C(11)-C(10)-C(7)	121.8(2)
C(10)-C(11)-C(12)	119.7(2)
O(13)-C(12)-C(11)	124.3(2)
O(13)-C(12)-C(9)	115.9(2)
C(11)-C(12)-C(9)	119.8(2)
C(12)-O(13)-C(14)	117.34(18)
O(1S)-S(1S)-C(1S)	106.10(13)
O(1S)-S(1S)-C(2S)	106.34(12)
C(1S)-S(1S)-C(2S)	97.25(13)

Table 10-3 : Bond lengths [\AA] and angles [deg] for **172**.

	U11	U22	U33	U23	U13	U12
S(1)	31(1)	26(1)	42(1)	-6(1)	9(1)	-1(1)
O(2)	33(1)	46(1)	59(1)	-12(1)	11(1)	2(1)
C(2)	33(1)	30(1)	39(1)	3(1)	7(1)	1(1)
N(3)	32(1)	29(1)	39(1)	2(1)	7(1)	-4(1)
O(4)	48(1)	34(1)	51(1)	-11(1)	7(1)	-7(1)
C(4)	40(1)	24(1)	37(1)	3(1)	6(1)	-3(1)
C(5)	36(1)	22(1)	32(1)	0(1)	6(1)	-1(1)
C(6)	40(1)	24(1)	35(1)	-1(1)	8(1)	1(1)
C(7)	38(1)	25(1)	35(1)	2(1)	10(1)	3(1)
C(8)	43(1)	29(1)	36(1)	-1(1)	12(1)	4(1)
C(9)	43(1)	33(1)	41(1)	0(1)	16(1)	10(1)
C(10)	36(1)	31(1)	45(1)	-6(1)	13(1)	2(1)
C(11)	36(1)	31(1)	45(1)	-4(1)	10(1)	1(1)
C(12)	35(1)	31(1)	41(1)	9(1)	13(1)	5(1)
O(13)	34(1)	41(1)	51(1)	3(1)	14(1)	5(1)
C(14)	35(1)	36(1)	47(1)	7(1)	6(1)	0(1)
S(1S)	29(1)	37(1)	39(1)	-1(1)	8(1)	-1(1)
O(1S)	37(1)	50(1)	57(1)	-1(1)	6(1)	-17(1)
C(1S)	30(1)	49(1)	71(2)	-7(1)	14(1)	4(1)
C(2S)	40(1)	46(1)	51(1)	-9(1)	9(1)	4(1)

Table 10-4 : Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 172. The anisotropic displacement factor exponent takes the form: $-2 \pi^2 [h^2 a^{*2} U11 + \dots + 2 h k a^* b^* U12]$

	x	y	z	U(eq)
H(3)	3360	9344	1662	40
H(6)	910	10111	3211	39
H(8)	-451	10434	3497	43
H(9)	-1865	10049	2877	45
H(10)	-39	7766	396	44
H(11)	-1459	7384	-271	44
H(14A)	-2848	7995	-1630	59
H(14B)	-3596	7736	-619	59
H(14C)	-2759	6989	-179	59
H(1S1)	6170	34	3959	74
H(1S2)	6435	940	2599	74
H(1S3)	6192	-349	1963	74
H(2S1)	4437	2072	3613	68
H(2S2)	5375	2412	3526	68
H(2S3)	5198	1438	4880	68

Table 10-5 : Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **172**.

10.2 X-Ray Crystal Structure of 179.

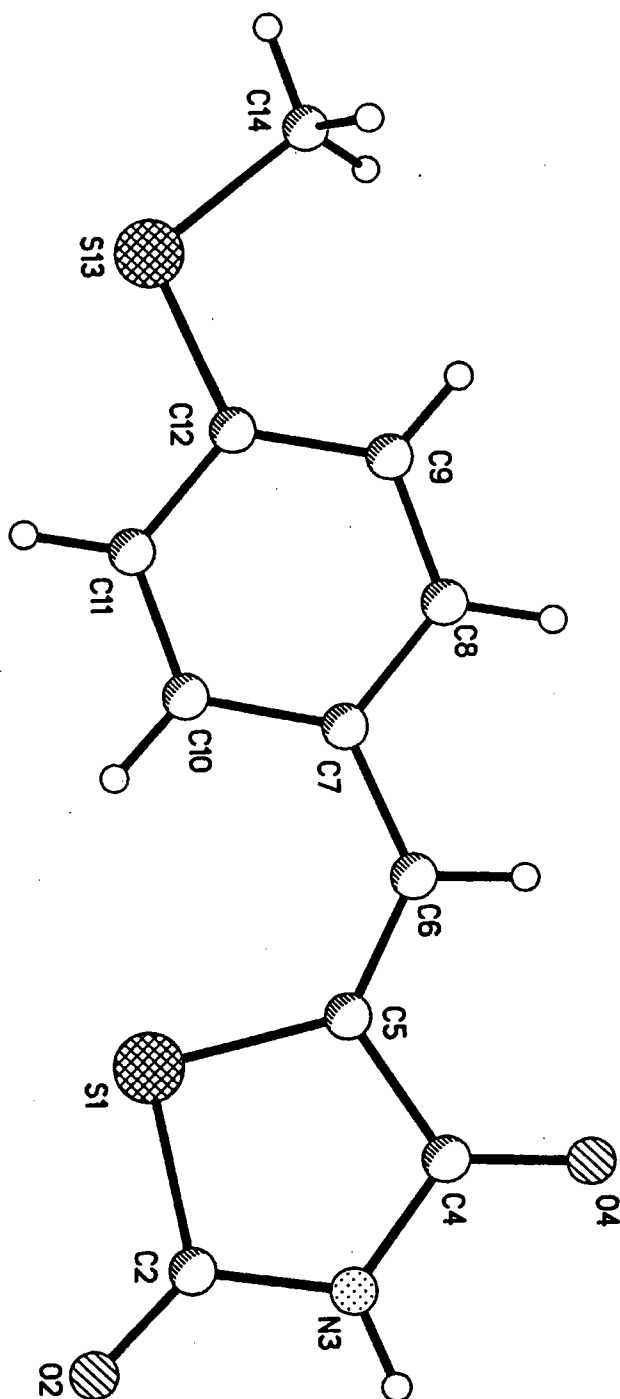
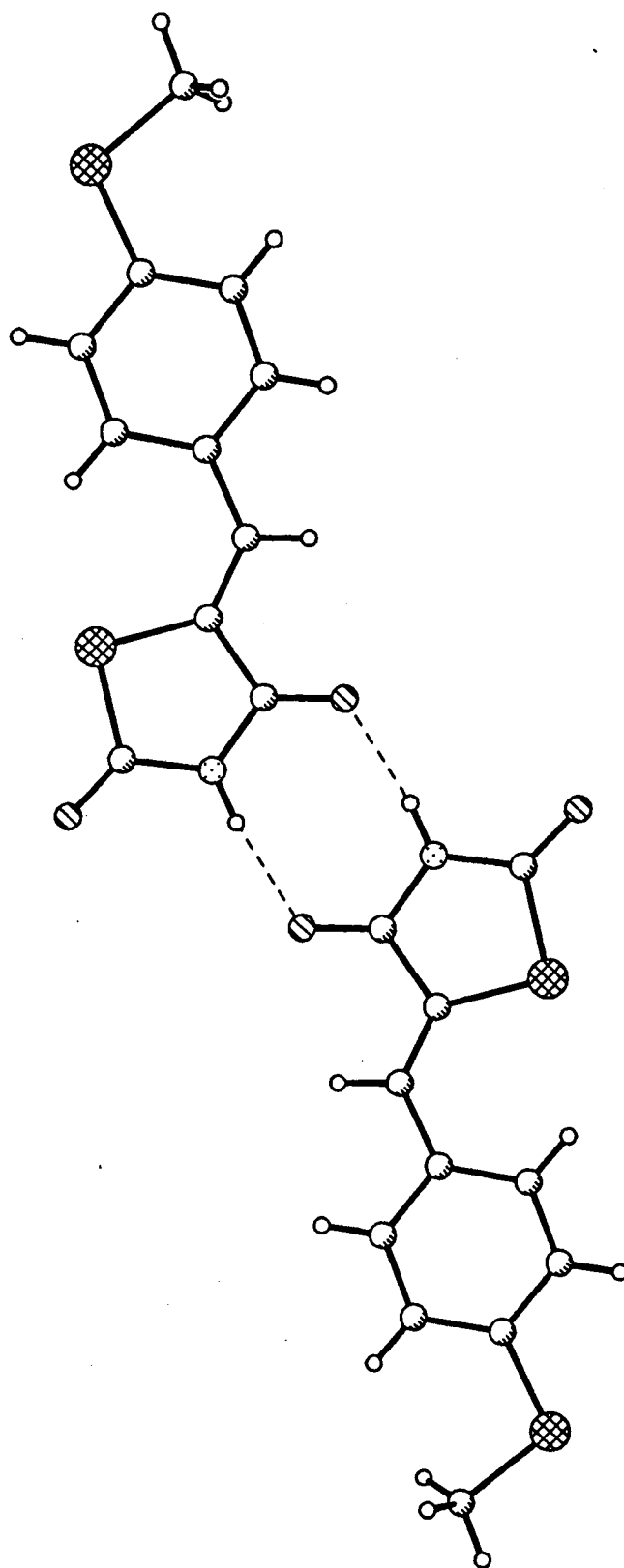


Fig. 10-2 : Representation of the Structure of 179.

Fig. 10-3 : Representation of the Structure of 179 Featuring Hydrogen Bonding Interactions.



A. CRYSTAL DATA

Empirical formula	$C_{11}H_9NO_2S_2$
Formula weight	251.31
Wavelength	1.54178 Å
Temperature	150(2) K
Crystal system	Monoclinic
Space group	P21/c
Unit cell dimensions	a = 6.5676(6) Å alpha = 90 deg. b = 9.8187(12) Å beta = 100.089(10) deg. c = 17.6542(18) Å gamma = 90 deg.
Volume	1120.8(2) Å ³
Number of reflections for cell	82 (20 < theta < 22 deg.)
Z	4
Density (calculated)	1.489 Mg/m ³
Absorption coefficient	4.181 mm ⁻¹
F(000)	520

B. DATA COLLECTION

Crystal description	Yellow needle
Crystal size	0.32 x 0.16 x 0.11 mm
Theta range for data collection	5.09 to 69.88 deg.
Index ranges	-6<=h<=7, -9<=k<=11, -16<=l<=21
Reflections collected	3492
Independent reflections	1995 [R(int) = 0.0149]
Scan type	Omega-theta
Absorption correction	Optimised Numerical (T _{min} = 0.657, T _{max} = 0.368)

C. SOLUTION AND REFINEMENT.

Solution	direct methods (SHELXS-97)
Refinement type	Full-matrix least-squares on F^2
Program used for refinement	SHELXL-97
Hydrogen atom placement	geometric
Hydrogen atom treatment	riding
Data / restraints / parameters	1995/0/147
Goodness-of-fit on F^2	1.064
Conventional R [$F > 4\sigma(F)$]	$R_1 = 0.0288$ [1787 data]
Weighted R (F^2 and all data)	$wR_2 = 0.0756$
Extinction coefficient	0.0018(3)
Final maximum delta/sigma	0.001
Weighting scheme	
calc $w^{-1} = [\sigma^2(F_o^2) + (0.0362P)^2 + 0.5453P]$ where $3P = (F_o^2 + 2F_c^2)$	
Largest diff. peak and hole	0.229 and -0.222 e. \AA^{-3}

Table 10-6 : Crystal data and structure refinement for **179**.

	x	y	z	U(eq)
S(1)	10572(1)	3253(1)	4812(1)	38(1)
O(2)	14167(2)	3635(2)	5733(1)	61(1)
C(2)	13108(3)	2863(2)	5307(1)	41(1)
N(3)	13634(2)	1566(1)	5126(1)	31(1)
O(4)	12532(2)	-304(1)	4397(1)	31(1)
C(4)	12222(3)	846(2)	4615(1)	26(1)
C(5)	10306(3)	1643(2)	4379(1)	27(1)
C(6)	8694(3)	1109(2)	3903(1)	27(1)
C(7)	6686(3)	1679(2)	3591(1)	26(1)
C(8)	5308(3)	868(2)	3090(1)	30(1)
C(9)	3386(3)	1337(2)	2743(1)	31(1)
C(10)	6032(3)	2999(2)	3741(1)	30(1)
C(11)	4116(3)	3468(2)	3407(1)	31(1)
C(12)	2763(3)	2647(2)	2901(1)	28(1)
S(13)	376(1)	3377(1)	2505(1)	36(1)
C(14)	-933(3)	2081(2)	1889(1)	41(1)

Table 10-7 : Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **179**. $U(\text{eq})$ is defined as one third of the trace of the orthogonalized U_{ij} tensor.

S(1)-C(5)	1.7510(17)
S(1)-C(2)	1.781(2)
O(2)-C(2)	1.201(2)
C(2)-N(3)	1.373(2)
N(3)-C(4)	1.370(2)
O(4)-C(4)	1.221(2)
C(4)-C(5)	1.479(2)
C(5)-C(6)	1.338(2)
C(6)-C(7)	1.450(2)
C(7)-C(8)	1.398(2)
C(7)-C(10)	1.405(2)
C(8)-C(9)	1.382(2)
C(9)-C(12)	1.392(2)
C(10)-C(11)	1.373(2)
C(11)-C(12)	1.400(2)
C(12)-S(13)	1.7542(17)
S(13)-C(14)	1.792(2)
C(5)-S(1)-C(2)	91.92(8)
O(2)-C(2)-N(3)	126.18(18)
O(2)-C(2)-S(1)	124.15(15)
N(3)-C(2)-S(1)	109.67(13)
C(4)-N(3)-C(2)	117.61(15)
O(4)-C(4)-N(3)	123.61(15)
O(4)-C(4)-C(5)	125.50(15)
N(3)-C(4)-C(5)	110.89(14)
C(6)-C(5)-C(4)	120.68(15)
C(6)-C(5)-S(1)	129.45(13)
C(4)-C(5)-S(1)	109.87(12)
C(5)-C(6)-C(7)	130.86(15)
C(8)-C(7)-C(10)	117.35(15)
C(8)-C(7)-C(6)	117.72(15)
C(10)-C(7)-C(6)	124.92(15)
C(9)-C(8)-C(7)	122.01(16)
C(8)-C(9)-C(12)	119.78(16)
C(11)-C(10)-C(7)	121.08(16)
C(10)-C(11)-C(12)	120.82(16)
C(9)-C(12)-C(11)	118.95(16)
C(9)-C(12)-S(13)	124.84(13)
C(11)-C(12)-S(13)	116.21(13)
C(12)-S(13)-C(14)	104.56(9)

Table 10-8 : Bond lengths [\AA] and angles [deg] for 179.

	U11	U22	U33	U23	U13	U12
S(1)	32(1)	33(1)	44(1)	-13(1)	-7(1)	9(1)
O(2)	47(1)	49(1)	74(1)	-28(1)	-25(1)	11(1)
C(2)	36(1)	38(1)	43(1)	-9(1)	-7(1)	8(1)
N(3)	26(1)	31(1)	34(1)	-2(1)	-5(1)	5(1)
O(4)	28(1)	27(1)	36(1)	-1(1)	-2(1)	5(1)
C(4)	24(1)	29(1)	25(1)	3(1)	2(1)	2(1)
C(5)	25(1)	28(1)	27(1)	1(1)	4(1)	5(1)
C(6)	27(1)	26(1)	28(1)	1(1)	5(1)	2(1)
C(7)	23(1)	27(1)	28(1)	1(1)	4(1)	2(1)
C(8)	26(1)	27(1)	38(1)	-3(1)	5(1)	0(1)
C(9)	24(1)	31(1)	36(1)	-2(1)	1(1)	-3(1)
C(10)	26(1)	28(1)	33(1)	-3(1)	-2(1)	0(1)
C(11)	28(1)	25(1)	38(1)	2(1)	1(1)	3(1)
C(12)	24(1)	28(1)	32(1)	7(1)	3(1)	-2(1)
S(13)	26(1)	27(1)	50(1)	9(1)	-7(1)	-1(1)
C(14)	31(1)	43(1)	44(1)	5(1)	-8(1)	-6(1)

Table 10-9 : Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **179**. The anisotropic displacement factor exponent takes the form: $-2 \pi^2 [h^2 a^{*2} U11 + \dots + 2 h k a^* b^* U12]$

	x	y	z	U(eq)
H(4)	14834	1209	5329	38
H(6)	8901	204	3742	32
H(8)	5704	-34	2986	36
H(9)	2494	769	2397	37
H(10)	6930	3576	4080	36
H(11)	3703	4362	3520	37
H(14A)	-118	1851	1492	62
H(14B)	-2296	2415	1645	62
H(14C)	-1099	1268	2194	62

Table 10-10 : Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **179**

10.3 X-Ray Crystal Structure of 181.

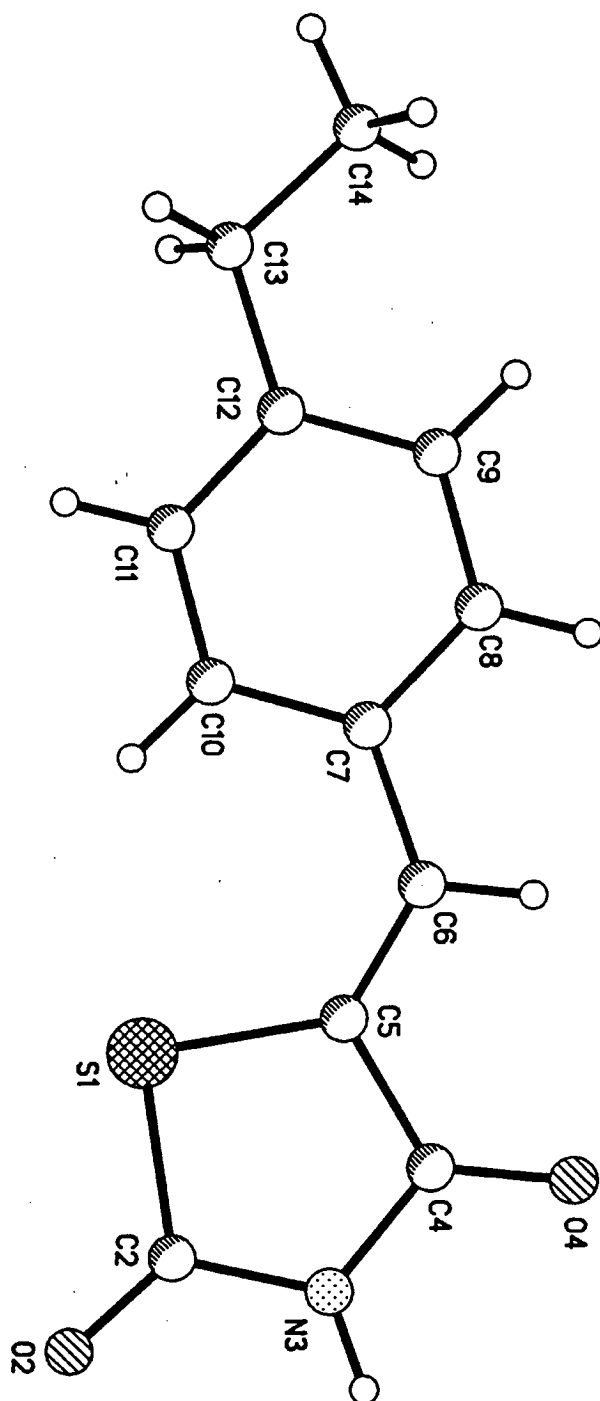


Fig. 10-4 : Representation of the Structure of 181.

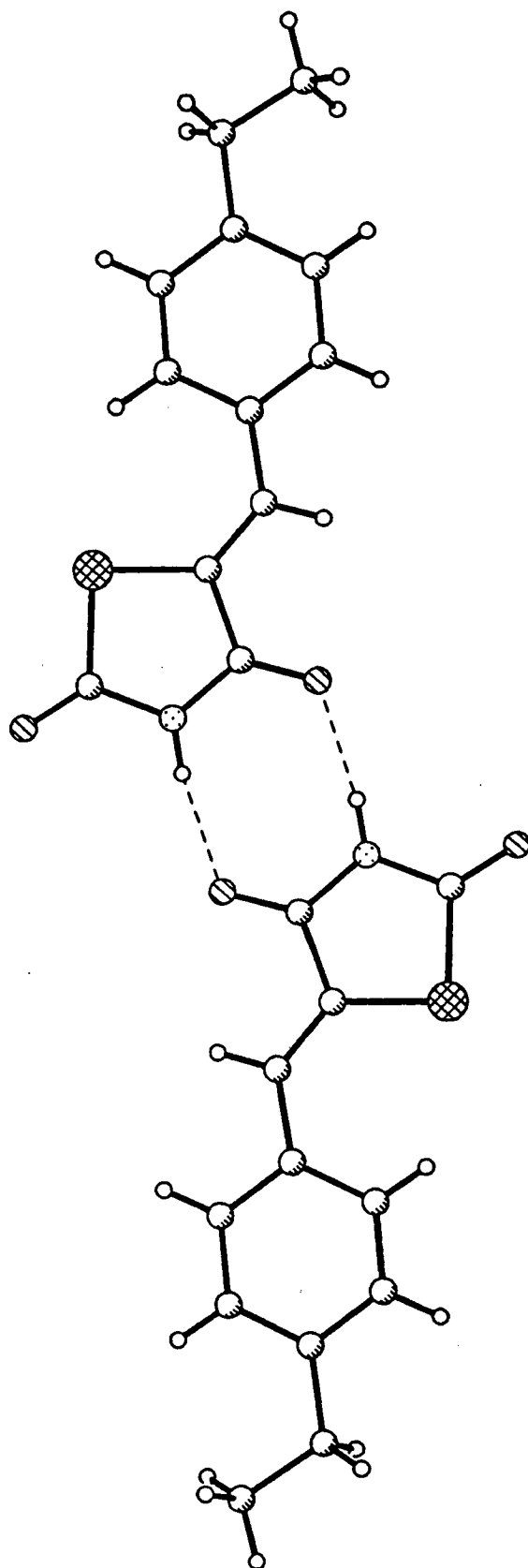


Fig. 10-5 : Representation of the Structure of 181 Featuring Hydrogen Bonding Interactions.

A. CRYSTAL DATA

Empirical formula	$C_{12}H_{11}NO_2S$
Formula weight	233.28
Wavelength	1.54178 Å
Temperature	150(2) K
Crystal system	Monoclinic
Space group	P21/c
Unit cell dimensions	a = 11.7287(12) Å alpha = 90 deg. b = 11.6561(11) Å beta = 90.576(12) deg. c = 7.8628(11) Å gamma = 90 deg.
Volume	1074.9(2) Å ³
Number of reflections for cell	92 (20 < theta < 22 deg.)
Z	4
Density (calculated)	1.442 Mg/m ³
Absorption coefficient	2.544 mm ⁻¹
F(000)	488

B. DATA COLLECTION

Crystal description	Colourless plate developed in (100)
Crystal size	0.54 x 0.39 x 0.12 mm
Theta range for data collection	3.77 to 70.04 deg.
Index ranges	-11 ≤ h ≤ 14, -14 ≤ k ≤ 14, -9 ≤ l ≤ 8
Reflections collected	4463
Independent reflections	1950 [R(int) = 0.0281]
Scan type	Omega-theta
Absorption correction	Psi-scans (T _{min} = 0.421, T _{max} = 0.825)

C. SOLUTION AND REFINEMENT.

Solution	direct methods (SHELXS-97)
Refinement type	Full-matrix least-squares on F^2
Program used for refinement	SHELXL-97
Hydrogen atom placement	Geometric (after difference map)
Hydrogen atom treatment	Riding or rotating group
Data / restraints / parameters	1950/0/147
Goodness-of-fit on F^2	1.057
Conventional R [$F > 4\sigma(F)$]	$R_1 = 0.0321$ [1831 data]
Weighted R (F^2 and all data)	$wR_2 = 0.0875$
Extinction coefficient	0.0039(6)
Final maximum delta/sigma	0.001
Weighting scheme calc $w^{-1} = [\sigma^2(F_o^2) + (0.0485P)^2 + 0.5297P]$ where $3P = (F_o^2 + 2F_c^2)$	
Largest diff. peak and hole	0.299 and -0.265 e. \AA^{-3}

Table 10-11 : Crystal data and structure refinement for **181**.

	x	y	z	U(eq)
S(1)	2113(1)	7790(1)	875(1)	21(1)
O(2)	4018(1)	7358(1)	2606(2)	31(1)
C(2)	3543(1)	7982(1)	1610(2)	21(1)
N(3)	4005(1)	8945(1)	885(2)	20(1)
O(4)	3675(1)	10378(1)	-1044(1)	24(1)
C(4)	3345(1)	9536(1)	-263(2)	19(1)
C(5)	2195(1)	9021(1)	-411(2)	18(1)
C(6)	1396(1)	9510(1)	-1402(2)	19(1)
C(7)	227(1)	9177(1)	-1793(2)	18(1)
C(8)	-399(1)	9869(1)	-2903(2)	21(1)
C(9)	-1515(1)	9606(1)	-3366(2)	22(1)
C(10)	-318(1)	8203(1)	-1135(2)	21(1)
C(11)	-1427(1)	7947(1)	-1604(2)	22(1)
C(12)	-2050(1)	8638(1)	-2728(2)	19(1)
C(13)	-3258(1)	8306(1)	-3191(2)	26(1)
C(14)	-3844(1)	9042(2)	-4530(2)	31(1)

Table 10-12 : Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **181**. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

S(1)-C(5)	1.7579(15)
S(1)-C(2)	1.7835(16)
O(2)-C(2)	1.202(2)
C(2)-N(3)	1.373(2)
N(3)-C(4)	1.369(2)
O(4)-C(4)	1.2221(18)
C(4)-C(5)	1.481(2)
C(5)-C(6)	1.340(2)
C(6)-C(7)	1.454(2)
C(7)-C(8)	1.393(2)
C(7)-C(10)	1.404(2)
C(8)-C(9)	1.390(2)
C(9)-C(12)	1.388(2)
C(10)-C(11)	1.381(2)
C(11)-C(12)	1.397(2)
C(12)-C(13)	1.510(2)
C(13)-C(14)	1.518(2)
C(5)-S(1)-C(2)	91.58(7)
O(2)-C(2)-N(3)	125.70(14)
O(2)-C(2)-S(1)	124.33(12)
N(3)-C(2)-S(1)	109.97(11)
C(4)-N(3)-C(2)	117.55(12)
O(4)-C(4)-N(3)	123.72(13)
O(4)-C(4)-C(5)	125.40(14)
N(3)-C(4)-C(5)	110.89(12)
C(6)-C(5)-C(4)	120.31(13)
C(6)-C(5)-S(1)	129.76(12)
C(4)-C(5)-S(1)	109.93(11)
C(5)-C(6)-C(7)	131.41(14)
C(8)-C(7)-C(10)	117.41(14)
C(8)-C(7)-C(6)	117.87(13)
C(10)-C(7)-C(6)	124.72(14)
C(9)-C(8)-C(7)	121.73(14)
C(12)-C(9)-C(8)	120.85(14)
C(11)-C(10)-C(7)	120.49(14)
C(10)-C(11)-C(12)	122.01(14)
C(9)-C(12)-C(11)	117.51(14)
C(9)-C(12)-C(13)	123.21(14)
C(11)-C(12)-C(13)	119.28(14)
C(12)-C(13)-C(14)	116.10(14)

Table 10-13 : Bond lengths [\AA] and angles [deg] for **181**.

	U11	U22	U33	U23	U13	U12
S(1)	16(1)	21(1)	26(1)	5(1)	-2(1)	-3(1)
O(2)	21(1)	36(1)	35(1)	15(1)	-4(1)	-1(1)
C(2)	18(1)	24(1)	21(1)	2(1)	1(1)	0(1)
N(3)	16(1)	22(1)	21(1)	1(1)	-2(1)	-3(1)
O(4)	21(1)	22(1)	29(1)	5(1)	-3(1)	-5(1)
C(4)	18(1)	19(1)	19(1)	-3(1)	0(1)	-1(1)
C(5)	17(1)	16(1)	20(1)	-1(1)	1(1)	-2(1)
C(6)	20(1)	17(1)	19(1)	0(1)	1(1)	-2(1)
C(7)	18(1)	18(1)	18(1)	-2(1)	0(1)	1(1)
C(8)	23(1)	19(1)	22(1)	1(1)	0(1)	-2(1)
C(9)	22(1)	22(1)	22(1)	2(1)	-4(1)	2(1)
C(10)	19(1)	21(1)	24(1)	3(1)	-4(1)	0(1)
C(11)	21(1)	20(1)	27(1)	2(1)	-1(1)	-3(1)
C(12)	17(1)	22(1)	19(1)	-4(1)	-2(1)	1(1)
C(13)	18(1)	29(1)	29(1)	0(1)	-5(1)	-2(1)
C(14)	23(1)	34(1)	35(1)	-1(1)	-10(1)	2(1)

Table 10-14 : Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **181**. The anisotropic displacement factor exponent takes the form: $-2 \pi^2 [h^2 a^{*2} U11 + \dots + 2 h k a^* b^* U12]$

	x	y	z	U(eq)
H(3)	4700	9174	1148	24
H(6)	1635	10196	-1945	22
H(8)	-54	10539	-3355	25
H(9)	-1917	10095	-4130	26
H(10)	80	7716	-363	25
H(11)	-1776	7281	-1148	27
H(13A)	-3721	8329	-2145	31
H(13B)	-3254	7503	-3599	31
H(14A)	-3905	9832	-4114	46
H(14B)	-4609	8738	-4765	46
H(14C)	-3397	9032	-5577	46

Table 10-15 : Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **181**

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